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Edited by

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N-15 SIGNALS OF NITROGEN SOURCE AND FATE IN A SEMIARID WETLAND

Jeffrey M. Heikoop, Donald D. Hickmott, Danny Katzman, Patrick Longmire and Robert S. Beers (Los Alamos National Laboratory, Los Alamos, New Mexico)

ABSTRACT: Nitrogen isotope measurements of plants and waters are being used to identify nitrogen fluxes and transformations occurring in a natural cattail marsh in Sandia Canyon at Los Alamos National Laboratory (LANL). The marsh receives inputs of treated sewage wastewater, containing nitrate enriched in ^{15}N ($\delta^{15}N$ of 32.4 ‰, concentrations up to 30 mg/L). Cattails growing near the head of the primary wetland have $\delta^{15}N$ values up to 37.8 ‰, suggesting that sewage nitrate is their main source of nitrogen with in-situ denitrification leading to further enrichment of the wetland nitrate pool. These cattails also have lower molar C/N ratios, relative to other cattails in the system, with values around 16. These results suggest that nitrogen is both lost from the system via denitrification and stored in plant material. Cattails growing near the distal end of the wetland, in more strongly reducing sediments that potentially have higher denitrification rates, were enriched in ^{13}C. This is due to decreased isotopic fractionation during uptake and assimilation of CO_2, resulting from partial closure of stomata under physiological redox stress. These cattails were enriched in ^{15}N, relative to cattails growing in central portions of the wetland. Natural abundance level isotopic measurements of wetland plants and waters could provide an effective technique to identify spatial gradients in nitrogen inputs, outputs, and transformation, in both natural and constructed wetlands.

INTRODUCTION

Biogeochemical cycling of nitrogen in wetlands is an important factor in ecosystem structure and function, water quality and long-term sequestration of fixed nitrogen. Treatment wetlands, both natural and constructed, are effective systems at naturally attenuating nitrogen inputs. Nitrogen and carbon isotopic measurements of wetland plants and waters can potentially be used to identify nitrogen sources to wetlands (e.g. McClelland et al., 1997; McClelland, J.W. and Valiela, I., 1998), and to identify spatial gradients in nitrogen attenuating processes (see Kendall et al., 1997).

Various anthropogenic sources of nitrogen input to wetlands have distinct nitrogen isotope signatures. The ratio of the heavy isotope of nitrogen, ^{15}N, to the light isotope of nitrogen, ^{14}N, is reported as $\delta^{15}N$, where;

$$\delta^{15}N = ((R_{sample}/R_{standard})-1)*1000); R={}^{15}N/{}^{14}N; \text{units of ‰}.$$

The international standard is atmospheric N_2. Fertilizer nitrogen typically has values close to 0 ‰, whereas manure/sewage nitrogen typically has values of 10-

20 ‰ or higher (Heaton, 1986). Nitrogen from atmospheric deposition (NO_X, NH_3^+, organic nitrogen) may have values less than 0 ‰. Nitrogen transforming processes, such as denitrification or ammonia volatilization, result in products enriched in ^{14}N. By mass balance, residual nitrogen source pools must be enriched in ^{15}N. Denitrification, for example, produces nitrogen gas that is up to 35 ‰ depleted relative to source nitrate, leaving residual nitrate pools highly enriched in ^{15}N (Heaton, 1986). Natural abundance level nitrogen isotope measurements have been used to identify sewage uptake by plants in freshwater and marine settings (e.g. Jordan et al., 1997; Costanzo et al, 2001), including ponds, marshes and coastal wetlands (McClelland et al., 1997; McClelland and Valiela, 1998; Cole et al., 2001; Heikoop et al., 2001). The United States Geological Survey is using plant-based isotope techniques to identify areas in the Everglades that are strongly reducing, where methylation of atmospheric mercury sources may occur (Kendall et al., 1997).

Carbon isotopes in plants have been used as an indicator of water stress. Plants under water stress partially close their stomata to avoid excessive losses of water as a result of transpiration. This limits exchange of CO_2 between internal plant pools of CO_2 and the external atmospheric pool. The net effect is that isotopic fractionation (preferential incorporation of ^{12}C) is reduced (Farquhar et al., 1982). Many wetland plants have a similar physiological response to redox stress in which they partially close their stomata under more strongly reducing conditions. *Typha domingensis*, for instance, has been shown to have significantly reduced stomatal conductance at an eH of -200 mV, while still maintaining relatively high rates of photosynthesis (Pezeshki et al., 1996). Carbon isotopes, therefore, are potentially useful in identifying redox gradients in wetlands that may be accompanied, for instance, by gradients in denitrification potential.

A small cattail marsh in Sandia Canyon, at Los Alamos National Laboratory (New Mexico, USA), receives inputs of nitrogen, mainly in the form of nitrate (concentrations up to 30 mg/L as nitrate), from Laboratory treated sewage wastewater. This wastewater is strongly enriched in ^{15}N, with $\delta^{15}N$ of 32.4 ‰ (Longmire, unpublished data). Background nitrate from meteoric precipitation has $\delta^{15}N$ of ~ 0 ‰ and concentrations of ~1 mg/L (Longmire, unpublished data). Waters discharged from the wetland have background concentrations of nitrate. The combination of isotopically distinct nutrient sources, and the presence of a nutrient attenuation gradient, make this an ideal setting to test the hypothesis that $\delta^{15}N$ and $\delta^{13}C$ of wetland plants reflect nutrient sources, and nutrient attenuating processes.

Site Description. The Sandia wetland is a ~ 1km long cattail (*Typha latifolia*) marsh developed on sediments deposited by runoff into the canyon during early (ca. 1950's) construction activities in the head of the watershed. These deposits, along with the stratigraphy of the underlying volcanic tuff, have resulted in a low gradient canyon reach suitable for wetland development. The climate is semi-arid and seasonally cold, with monsoonal rainfall occurring in July and August. Perennial water supply is provided by discharge from a wastewater treatment plant outfall. The outfall discharge is seasonally augmented by runoff from

snowmelt and monsoonal precipitation. In 1998 the outfall was relocated from a hillslope location at the head of the wetland to a location 0.4 km upcanyon. This has resulted in lowering of the water table and has possibly increased geomorphic instability in the upstream half of the wetland system, which had developed on terraces adjacent to the main channel. Several subsequent high-intensity flood events have caused incision and consequently partial dewatering of this upper portion of the wetland, resulting in reduced cattail growth. The downstream portion of the wetland (referred to as the primary wetland) remains perennially saturated and supports lush cattail growth.

MATERIALS AND METHODS

The upper 30 cm of cattail leaves were collected from cattails growing along the longitudinal axis (i.e. main surface water flow path) of the primary wetland. Additional samples were collected from the wetland periphery and from upstream terraces. Reference samples were collected from a nearby reservoir. Samples were homogenized to a fine powder and ~ 2-3 mg aliquots were analyzed by Elemental Analyzer – Continuous Flow – Isotope Ratio Mass Spectrometry, which provides N and C concentrations as well as $\delta^{15}N$ and $\delta^{13}C$ values. Precision (1 σ) on 6 replicate analyses was 0.2 ‰ for $\delta^{15}N$, 0.15 ‰ for $\delta^{13}C$; 0.7 % for [C] and, 0.07 % for [N].

RESULTS AND DISCUSSION

The average $\delta^{15}N$ of reference cattails and cattails from the longitudinal axis and periphery of the primary Sandia Canyon wetland are shown in Figure 1. Reference cattails have low $\delta^{15}N$, whereas cattails from the main longitudinal axis of the marsh are highly enriched in ^{15}N, with average values of ~25 ‰, reflecting the importance of sewage nitrate. Cattails from the periphery of the wetland have intermediate values reflecting a mixture of sewage and background nitrogen inputs. The trend in $\delta^{15}N$ versus distance along the longitudinal axis is shown in Figure 2. Several cattails near the head of the wetland are highly enriched in ^{15}N, with values up to ~38 ‰. These are the highest reported $\delta^{15}N$ values for plants that we are aware of. The high values reflect the high $\delta^{15}N$ of the sewage nitrate source plus further enrichment of the wetland nitrate pool resulting from in-situ denitrification.

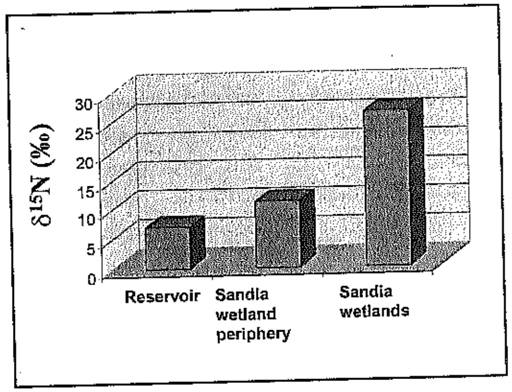

FIGURE 1. Mean $\delta^{15}N$ of reference cattails from a local reservoir, and of cattails from the Sandia Canyon wetland periphery and longitudinal axis.

The curvilinear trend in $\delta^{15}N$ (Figure 2) is believed to reflect the decreasing importance of ^{15}N-enriched sewage nitrate with distance downstream along the longitudinal axis of the wetland, countered by increasing rates of denitrification in more reducing downstream sediments. Sewage nitrate will be taken up by cattails at the head of the wetland and will also be eliminated by denitrification in waterlogged sediments. Sediments are reducing throughout the wetland, but the upstream portion of the wetland is characterized by a mix of gravel and organic matter (eH of +188 mV), while downstream sediments are composed of more strongly reducing organic-rich mud (eH of –240 mV).

The trend in molar C/N ratios is shown in Figure 3. Cattails at the head of the wetland have the highest N contents, suggesting that plant uptake is a direct factor in sewage wastewater nitrogen attenuation, in addition to the influence of denitrification mentioned previously.

Cattail $\delta^{13}C$ increases with distance downstream (Figure 4). $\delta^{13}C$ of atmospheric CO_2 will be constant over the length of the wetland. Since the wetland soils are saturated along the entire length of the primary wetland, the $\delta^{13}C$ signal is most likely a function of redox stress. In this sense, with detailed laboratory and field calibration, $\delta^{13}C$ of wetland plants could potentially be developed as a redox proxy, which could in turn be used to infer redox gradients that might be responsible for gradients in denitrification potential within wetlands. Plant-based $\delta^{13}C$ records could potentially be used to deconvolute the contributions of denitrification from nutrient source signals to $\delta^{15}N$ of wetland plants.

The few remaining relict cattails growing on a dewatered upstream terrace also had higher $\delta^{13}C$, relative to cattails growing in the adjacent channel (-26 = versus –27.6 ‰, $p<0.01$). In this case, the higher $\delta^{13}C$ is due to water stress as

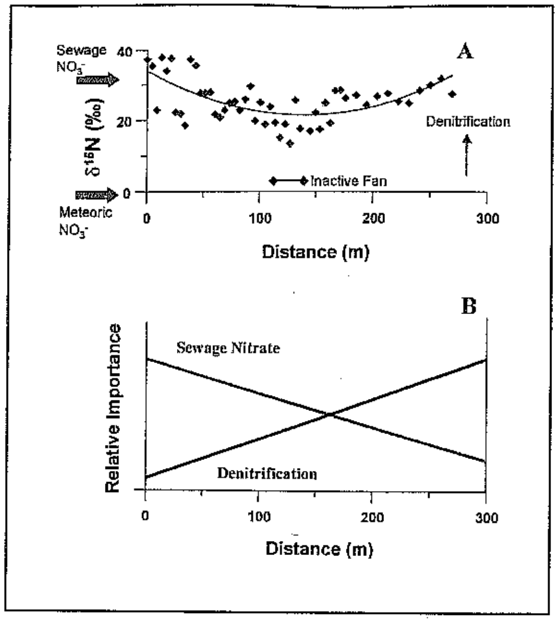

FIGURE 2. A. $\delta^{15}N$ of cattails versus distance along the longitudinal axis of the primary wetland. Values for sewage and meteoric nitrate are shown, as is the isotopic effect of denitrification. The curvilinear trend is significant at $p<0.001$, with R^2 = 0.40. Location of an inactive alluvial fan is shown. This fan may preferentially deliver surface and subsurface side slope flow (with low $\delta^{15}N$ of nitrate) to the wetland. Groundwater has been observed at levels higher than the wetland, in holes augured into this fan following precipitation events. B. Qualitative model explaining the trend in cattail $\delta^{15}N$ (see text).

opposed to redox stress. Since higher eH values will tend to occur in less saturated soils, the physiological effects of water stress on carbon isotope fractionation during photosynthesis could mask $\delta^{13}C$ signals associated with redox conditions. Plant-based $\delta^{13}C$ redox signals, therefore, will be best applied in wetland soils that are constantly saturated during plant growing seasons. $\delta^{15}N$ of cattails growing on this upstream terrace have values intermediate to sewage and meteoric nitrogen sources. The main source of new nitrogen will be meteoric inputs (the cattails are rooted well above the current water table), but mineralization of older (prior to relocation of the outfall) cattail detritus would provide a source of recycled heavy nitrogen. When alive, the older cattails would have been at the head of a more extensive wetland receiving sewage input from the hillslope outfall, prior to its being moved upstream.

Combined, these results allow us to develop a simple conceptual model of nitrogen sources, sinks and transformations occurring in the primary Sandia wetland (Figure 5). New nitrogen will be delivered to the subsurface rooting zone via diffusion of nitrate from surface waters. Both plant uptake and denitrification will reduce nitrate levels in the subsurface, creating this diffusion gradient (Mitsch and Gosselink, 1993). Significant transport of nitrate in alluvial groundwater is unlikely for these same reasons. There are only low levels of ammonia in outfall waters, and horizontal

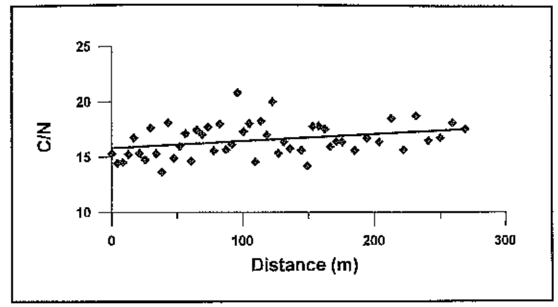

FIGURE 3. Molar C/N ratios of cattails versus distance along the longitudinal axis of the primary wetland; $p=0.02$, $R^2=0.11$.

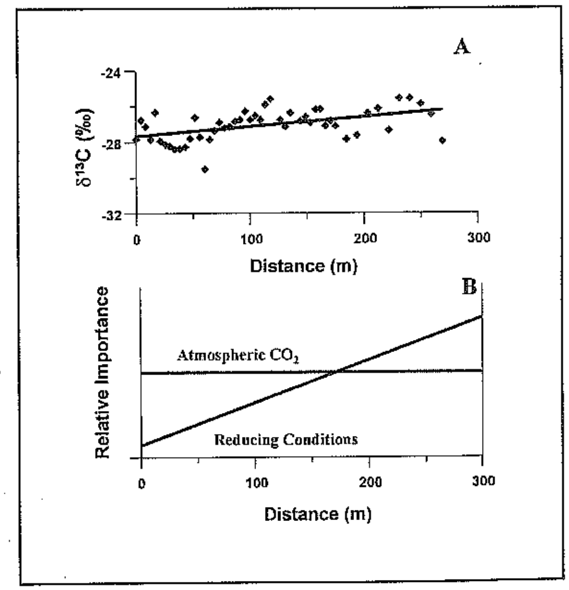

FIGURE 4. A. $\delta^{13}C$ of cattails versus distance along the longitudinal axis of the primary wetland; $p=0.001$, $R^2=0.23$. B. Qualitative model explaining trend in $\delta^{13}C$ (see text).

advection of ammonia in groundwaters is unlikely due to immobilization of ammonia on negatively charged soil particles (Mitsch and Gosselink, 1993). Ammonia, therefore, is likely to be recycled close to organic detritus from which it is derived.

Nitrate that is not attenuated within the wetland will be transported away from the wetland in surface waters, and to a lesser extent, in alluvial groundwaters. Nitrate that diffuses into the subsurface can be taken up by plants or can be denitrified and released as nitrogen gas or NO_2. We are also using nitrogen isotopes to assess the possibility that ^{15}N-enriched nitrate is being lost to underlying aquifers.

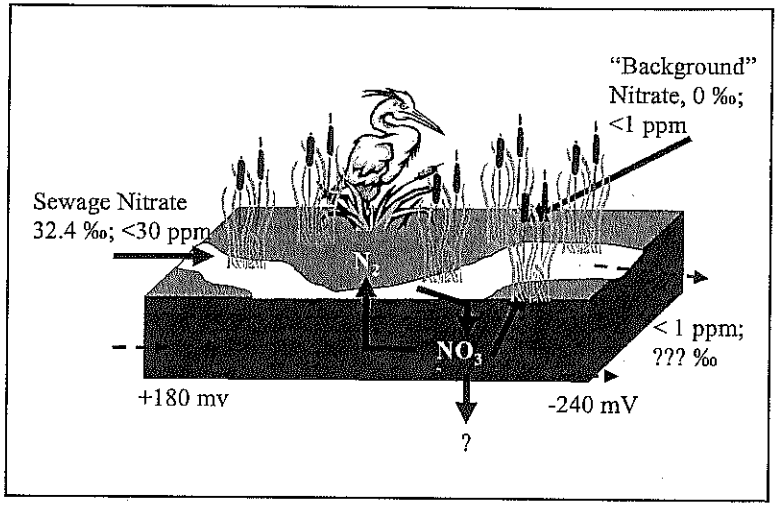

FIGURE 5. Conceptual model of nitrogen dynamics in Sandia wetland. Nitrogen source δ^{15}N and concentrations are shown, along with the prevailing redox gradient. Various nitrogen fluxes are illustrated including diffusion of nitrate into the subsurface, loss to underlying groundwaters, horizontal advection, plant uptake and denitrification.

The importance of new sewage nitrate is greatest towards the head of the wetland, while background sources become progressively more important downstream. The redox gradient within the wetland ensures that new nitrogen diffusing in from surface waters towards the end of the wetland is more completely denitrified, leading to greater isotopic enrichment of the residual subsurface nitrate pool available for plant uptake. Plant nitrogen contents are highest at the head of the wetland where nitrate-enriched treated sewage wastewater first enters the system. Despite significant downstream trends, there is considerable unexplained variability in δ^{15}N, δ^{13}C and C/N ratios. This likely reflects the heterogeneous nature of wetland sediments, hydrology, rooting depths etc.

These concepts are being investigated further by studying the Martin Canyon wetland at LANL. This wetland receives inputs of nitrate derived from high explosives. This nitrate presumably has low δ^{15}N as fertilizer nitrate is used in the production of high explosives. Nitrate levels in this wetland (e.g. 0.51 mg/L) are significantly lower than nitrate levels in Martin Spring (e.g. 15.2 mg/l). Martin Spring is located upstream of the wetland and is the primary source of water in the canyon. This wetland was burned during the Cerro Grande Fire in May 2000, affording an opportunity to study nutrient dynamics during recovery. Though new nitrogen sources (high explosive nitrate and background meteoric nitrogen) will likely not be distinct isotopically, denitrification could still impart an isotopic trend to cattails growing in the wetland.

CONCLUSIONS

Plant-based isotopic proxies of nitrogen sources, and nitrogen transforming processes, could provide a valuable additional tool for the study of nutrient dynamics in natural and treatment wetlands in which inorganic nitrogen attenuation is a goal. This technique will need to be carefully calibrated in individual wetlands, but could supplement traditional instrumental techniques and water-based measurements. Each plant in a wetland can be thought of as a monitoring well sampling the sediments in which it is growing. If the chemistry of a wetland plant faithfully reflects the sediment porewater chemistry, then valuable information can be derived from the plants themselves. This information can be garnered with simple, quick field sampling and with easy, relatively inexpensive analytical techniques. Plant-based chemical information can be obtained without disturbing wetland sediments, and hence wetland function. The signals obtained are time-integrated, thus smoothing out high frequency temporal variations. Data can be obtained at whatever spatial precision is desired. Properly developed, these techniques could help us to monitor spatial variation in treatment wetland performance, and to monitor this performance through time, helping us to see inside the "black box" with non-invasive techniques.

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