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Stable isotope dynamics of nitrogen sewage effluent uptake in a semi-arid wetland

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Stable isotope dynamics of nitrogen sewage effluent uptake in a semi-arid wetland showed high N variability at multiple trophic levels.

Abstract

Our objectives were to determine (1) how much N is transferred into the food web via plants from a wetland receiving not only inputs of treated sewage effluent, but also containing contaminants such as polychlorinated biphenyls (PCBs), (2) how birds, as consumers, utilize exogenous N and uptake PCBs in relation to the food web of the wetlands, (3) the feasibility of using isotopic analysis in estimating trophic levels in a semi-arid system. Our results demonstrate that there is very high spatial variability in the N isotopic composition of primary producers. Birds had lower variability in $\delta^{15}N$, despite feeding at multiple trophic levels. In very high spatial variability in $\delta^{15}N$ of primary producers, it is difficult to use N isotope techniques to define trophic levels relevant to the bioaccumulation of organic pollutants, but it is possible to track the flow of exogenous N through the food web.

Keywords: PCBs; Wetland; Stable isotope; Effluent; Birds; Nitrogen

1. Introduction

Nitrogen (N) inputs from anthropogenic sources can negatively impact the water quality of wetland ecosystems. Sandia wetland, at Los Alamos National Laboratory (LANL) in northern New Mexico, is an area of concern not only for treated effluent discharges, but also due to the presence of persistent contaminants such as polychlorinated biphenyls (PCBs). Previous studies at LANL have found detectable amounts of Aroclor-1260 in both small mammals and sediments (Bennett et al., 1999). Sandia wetland receives treated sewage effluent from a water treatment facility, leading to an increase of nitrogen (N) in the environment (though below US Environmental Protection Agency water quality standards of 10 ppm, nitrate as N). This N input is largely attenuated by plant uptake and denitrification as water flows through the wetland (Heikoop et al., 2002). Because this area is a wetland habitat, wildlife is more diverse and abundant than other nearby areas in the relatively arid landscape. This environment provides an opportunity to study the flow of N into food webs associated with the wetland and to assess the potential for bioaccumulation of PCBs.

Stable isotope analyses of N and carbon in tissues can provide valuable information on the diets of individuals and populations (Kelly, 2000). Organisms are typically enriched in heavy isotopes relative to their diet, leading to phrases such as "you are what you eat + 3.5‰" in the case of N isotopes. This reflects the fact that organisms excrete N enriched in ^{14}N, so by mass balance their bodies must be enriched in the heavy

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isotope ^{15}N (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). In the case of N isotopes, this enrichment, or fractionation factor, is ~3–4‰. When fractionation factors are well known, measuring the tissues of a consumer tells us the isotopic composition of its bulk, time-integrated diet. This technique, therefore, can be used to reconstruct food webs and hence potential pathways for contaminant bioaccumulation (e.g., Cabana and Rasmussen, 1994; Kidd et al., 1995; Kiriluk et al., 1995; Atwell et al., 1998). Fractionation factors are often well known for entire organisms, but may be less well known for individual tissues. Bird tissue N fractionation factors relative to diet were found to be between 3.7 and 5.6‰ in a study by Mizutani et al. (1992). Although the utility of stable isotopes has been identified there is still a need for laboratory and more controlled experiments to answer specific unknowns of stable isotopes and physiology (Bearhop et al., 2002). Uncertainties in this technique arise where such fractionation factors are not well constrained and where different primary producers in an ecosystem have access to multiple, isotopically distinct nutrient sources.

In most migratory passerine species of Europe and North America, adults replace body and flight feathers in the late summer immediately following the breeding season (Jenni and Winkler, 1994). This timing of molt patterns allows for newly molted feathers to be analyzed for stable isotopes on the breeding grounds where the feathers were grown. While this does not systematically allow for latitudinal differences in migration, such as wintering ground locations, to be analyzed since we chose newly molted feathers, it can provide stable isotope information on diet differences and trophic structure on the breeding grounds. This study was completed during this window of opportunity when new feathers are emerging and before migration. Birds that were banded during the breeding season in wetlands (June) were still located in the area when this study was completed (unpublished data). It was also completed in an isolated wetland in a canyon where local bird populations are congregated and assumed to have not left after breeding near the wetland.

Flooded wetland soils can present an ideal environment for denitrification of increased inputs of N. Denitrification occurs in strongly reducing environments such as waterlogged soils and can attenuate nitrate. Denitrification is also a strongly fractionating process in which residual nitrate is highly enriched in ^{15}N (Heaton, 1986). This denitrification signal can be recorded in wetland plants (Heikoop et al., 2002) and potentially promulgated up the food chain. Moreover, the isotopic composition of input N (e.g., sewage) may be isotopically distinct relative to background N sources (Heikoop et al., 2002). Identifying the relative importance of wetlands as permanent or temporary sinks of N can lead to the development of methods for optimizing the utilization of constructed or natural wetlands for wastewater treatment (Lund et al., 2000). Wetlands are often known to be N limited (Tilton, 1978), which could help compensate for an increase in N to the system. The isotopic signature of N can be used as a natural tracer to help quantify the flow of N in wetland systems and to assess the natural attenuation of N through processes such as denitrification.

Trophic transfer of persistent organics pollutants such as PCBs is the primary mechanism of biomagnification of contaminants in the environment. Carbon and N flow in plant and animal tissues can mirror the biomagnification of contaminants and can help to model the transport of contaminants through an ecosystem. This could be especially true in a more closed or segregated system such as a wetland in an arid landscape.

Our objectives were to estimate (1) how much N released into the wetland is transferred into the food web via plants, (2) how birds, as consumers, utilize exogenous N in relation to the food web of the wetlands, (3) to determine the concentration of PCBs in the blood of birds in the wetland, and (4) to determine the feasibility of using isotopic analysis to estimate trophic levels.

2. Materials and methods

This study was conducted from 13 to 23 August 2002 in upper Sandia Canyon, located on Los Alamos National Laboratory (LANL) property in Los Alamos County, New Mexico. The study site was located in a portion of the canyon that contains a wetland fed largely by sanitary effluent from a power plant and a sanitary wastewater system consolidation plant. The flow of the stream varies throughout the day due to differential water usage by LANL, though even at lowest levels there is always some above ground flow. The vegetation consists largely of broad-leaved cattail (*Typha latifolia*), with Gambel's oak (*Quercus gambelii*) and ponderosa pine (*Pinus ponderosa*) lining the drier walls of the canyon. Bird species composition can be somewhat delineated between these habitats, with certain species preferring the open cattail habitat and others staying within the cover of the oak/pine habitat. Due to incision in the wetland, there are open dry areas in the wetland that are dominated by sage (*Artemisia tridentata*) habitat.

Net sites were chosen to cover all three habitat types, though were concentrated in the wetland area as many birds utilize the stream to drink and bathe. Six mist-nets, five measuring 12 × 3 m and one measuring 6 × 3 m, were used over a 2 week period for a total of five netting days. Nets were placed every 25–50 m, and sites were moved approximately 5–10 m between days as capture rates decreased. Nets were opened at dawn (06:00 h mountain time) and closed between 10:00 h and 12:00 h, depending on activity level. Birds were brought back to a central processing station, where they were identified, aged, and sexed using measurements from Pyle (1997). Measurements of weight, flattened wing chord, and tarsus were taken, and the ninth primary feather was clipped and collected for stable isotope analysis. Bands were not placed on the birds, but because the ninth primary flight feather was clipped no birds that were recaptured were measured twice. The new molted feathers closest to the ninth primary feather were clipped at the top for the stable isotope analysis. Feathers were stored in bags and stored in a dark dry area until processing. A variety of plant species and particularly seeds and flowers were collected from the wetland. Some plant species were collected both in the direct wetland channel and at the wetland margin that was between 5 and 15 m away from the channel. Arthropods were collected with grasshopper sweep nets from 10:00–12:00 h. Samples were stored in plastic bags at −70 °C until processing. Samples were then oven dried for 24 h and ground to a powder for stable isotope analysis.

2.1. Isotope analysis

Feathers were washed in detergent and thoroughly rinsed to remove all oil, dirt, and residual detergent. A small piece (0.70–0.85 mg) of the distal feather was removed and wrapped in a tin capsule. The feather was then analyzed by elemental analysis (continuous flow-isotope ratio mass spectrometry utilizing a Finnigan MAT Delta plus XL located at the University of New Mexico). We verified that the technique provided precise values by comparing three sample runs from the same feather for three individuals and found close agreement

between the runs, with precision about 0.1‰ for carbon and 0.2‰ for nitrogen. Arthropod and vegetation samples were oven dried and a small amount (0.75–2.0 mg) was analyzed for δ^{13}C and δ^{15}N in the same manner as the feathers. Arthropods and vegetation were ground up after drying to a fine powder for isotope analysis.

2.2. PCB analysis

Analysis of PCB levels in blood was developed in humans to determine the extent of PCBs and organochlorines in communities of concern around the world (Gill et al., 1996). Recently this technique has been applied to birds to estimate the amount of exposure to PCBs and to compare locations of concern (Van den Brink and Bosveld, 2001). Quantification of PCB congeners is accomplished by capillary gas chromatography coupled with high-resolution mass spectrometry with electronic impact ionization (Van den Brink et al., 2000). Approximately 60–70= μl of heparinized blood was used in the analysis that were refrigerated.

2.3. Data analysis

The Statistical Analysis System (SAS Institute, Inc., 1987) was used for all statistical analyses, and assumptions for parametric statistics were examined. Carbon and N isotopes were compared amongst and between trophic levels and wetland locations with analysis of variance models (ANOVA). Means for each treatment (trophic level, locations) were compared with Duncan's multiple range test. Data not normally distributed or having unequal variances were compared with Kruskal–Wallis nonparametric tests. Slopes of heteroscedastic data for general trophic groups of birds were compared using nonparametric rank analysis of covariance.

Sewage N content in plants can be calculated using a simple mixing model:

$$\delta^{15}\text{N plant} = x\left(\delta^{15}\text{N sewage}\right) + (1-x)\left(\delta^{15}\text{N background}\right) \quad (1)$$

Background groundwater nitrate δ^{15}N values at LANL are as low as −2‰, so we have chosen that value here for δ^{15}N of background N inputs (Longmire, unpublished data) (x is a fraction between 0 and 1). Sewage nitrate has a value of 32.4‰, and Heikoop et al. (2002) measured nearly 38‰ in cattails with leaves. For our sewage end-member we chose a value of 34‰, which is close to the sewage input value, but allows for the possibility of in situ denitrification further enriching the sewage-derived nitrate within the wetland (see Heikoop et al., 2002). Given these values, then the percentage of sewage-derived nitrate taken up by the plants can be calculated as:

$$\%\ \text{sewage} = \frac{\delta^{15}\text{N plant} + 2}{36} \times 100 \quad (2)$$

This mixing model assumes no fractionation of nitrate during plant uptake.

The percentage sewage N in insects and birds can also be calculated in a similar fashion. We assigned a trophic level to each insect and bird based on its diet (see Table 1). Herbivores were assigned a value of 2, omnivores 2.5, and insectivores/carnivores a value of 3. We then assumed a trophic fractionation factor of 3.4‰ (an average value trophic enrichment noted in many studies (e.g., Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002) and corrected the raw δ^{15}N values as follows:

$$\text{corrected } \delta^{15}\text{N} = \text{raw } \delta^{15}\text{N} - ((\text{TL}-1)\times 3.4)) \quad (3)$$

Eq. (3) gives the average δ^{15}N of plants at the base of a particular insect or bird's food chain. The output from Eq. (3) can then be used in Eq. (2) to calculate the average percentage sewage nitrate being transferred to each consumer.

3. Results

Thirty-three birds from three main taxonomic families were netted and those that had newly molted feathers were analyzed for C and N stable isotopes (Table 2). There were also a total of 38 plants and 17 arthropods collected for analysis. Several plant species were collected at the treated water outfall at the top of the wetland and then collected further downstream. A total of 20 birds were analyzed for PCBs in the blood. No birds contained PCBs in detectable levels in the blood.

The total variation in δ^{15}N of each trophic level of individuals increased from plants to arthropods and then decreased for birds, but all trophic levels had heteroscedasticity ($F_{2,84} = 23.79$, $p < 0.0001$, Levene's test). The coefficient of variation for δ^{15}N in arthropods was 98%, for plants 68%, and for

Table 1
Avian species captured, migratory status, feeding zone, and diet of birds from Sandia wetland

Species	Status[a]	Feeding zone[b]	Diet[c]
Acorn Woodpecker, *Melanerpes fomicivorus*	R	B	I/O
American Robin, *Turdus migratorus*	R	G	I
Black-Billed Magpie, *Pica hudsonia*	R	G	I/O
Black-Headed Grosbeak, *Pheucticus melanocephalus*	M	F	I/O
Broad-Tailed Hummingbird, *Selasphorus platycercus*	M	F	N/I
Bush Tit, *Psaltriparus minimus*	R	F	I/O
Common Nighthawk, *Chordeiles minor*	M	A	I
Common Raven, *Corvus corax*	R	G	O
Great Horned Owl, *Bubo virginianus*	R	G	V
House Finch, *Carpodacus mexicanus*	R	G	S
House Wren, *Troglodytes aedon*	M	G	I
Lark Sparrow, *Chondestes grammacus*	M	G	S
MacGillivray's Warbler, *Oporornis tolmiei*	M	F	I
Orange-Crowned Warbler, *Vernivora celata*	M	F	I/O
Pine Siskin, *Carduelis pinus*	R	F/G	I
Western Bluebird, *Sialia mexicana*	R	G/A	I
Western Wood-Pewee, *Contopus sordidulus*	M	A	I

[a] M, migrant; R, resident.
[b] A, air; B, tree branch; G, ground; F, foliage.
[c] I, insects; N, nectar; O, omnivore; S, seeds; V, vertebrates.

Table 2
Mean stable-carbon and N isotope ratios of avian feathers in Sandia wetland

Species	n	δ^{13} C (SD)	δ^{15}N (SD)
Birds feathers			
Acorn Woodpecker, *Melanerpes fomicivorus*	1	−20.2	8.2
American Robin, *Turdus migratorus*	1	−20.1	11.5
Black-Billed Magpie, *Pica hudsonia*	1	−21.0	12.1
Black-Headed Grosbeak, *Pheucticus melanocephalus*	1	−22.2	7.4
Broad-Tailed Hummingbird, *Selasphorus platycercus*	2	−22.6 (1.2)	10.9 (2.5)
Bush Tit, *Psaltriparus minimus*	1	−20.5	6.5
Common Nighthawk, *Chordeiles minor*	1	−18.3	10.9
Common Raven, *Corvus corax*	1	−18.5	10.8
Great Horned Owl, *Bubo virginianus*	1	−22.4	10.1
House Finch, *Carpodacus mexicanus*	1	−20.3	8.8
House Wren, *Troglodytes aedon*	2	−20.9 (2.0)	11.9 (6.7)
Lark Sparrow, *Chondestes grammacus*	1	−22.5	11.4
MacGillivray's Warbler, *Oporornis tolmiei*	1	−21.0	9.3
Orange-Crowned Warbler, *Vernivora celata*	10	−22.1 (1.2)	6.6 (0.91)
Pine Siskin, *Carduelis pinus*	3	−22.1 (1.9)	7.6 (2.8)
Western Bluebird, *Sialia mexicana*	4	−19.9 (1.5)	8.6 (1.0)
Western Wood-Pewee, *Contopus sordidulus*	1	−20.7	8.6

birds 28%. Total percentage sewage N was log transformed for normality and there was no difference in the variability between groups ($F_{2,82} = 0.34$, $p = 0.71$, Levene's test). However, there were differences in average sewage N content between groups ($F_{2,82} = 8.87$, $p = 0.0003$) with birds having the lowest sewage content relative to plants and arthropods, which did not differ from each other. There was significant variability in raw δ^{15}N content between plants, arthropods, and birds ($F_{2,86} = 23.6$, $p < = 0.0001$, Levene's test). However, there were no differences in the raw δ^{15}N values between plants, arthropods, and birds ($\chi^2_{2,82} = 0.80$, $p = 0.67$, Kruskal–Wallis test).

There was no difference in the variability of δ^{13}C among groups ($F_{2,84} = 2.22$, $p = 0.12$, Levene's test). The values of δ^{13}C varied significantly between plants, arthropods, and birds with an increase with each level of 2.07‰ ($F_{2,85} = 47.02$, $p < = 0.001$). The slopes of the ratio of δ^{15}N to δ^{13}C (Figs. 1 and 2) did not differ between plants, arthropods, and birds ($F_{2,82} = 0.43$, $p = 0.65$, nonparameteric rank ANCOVA).

3.1. Avian isotopic signatures

Feather δ^{13}C values showed less variation compared to δ^{15}N values (Table 2). There was no difference in δ^{13}C values in feathers between the three trophic groupings (herbivore/nectar feeding, omnivore, insectivore/carnivore) ($F_{2,30} = 2.08$, $p = 0.14$). Most of the feather δ^{13}C values are consistent

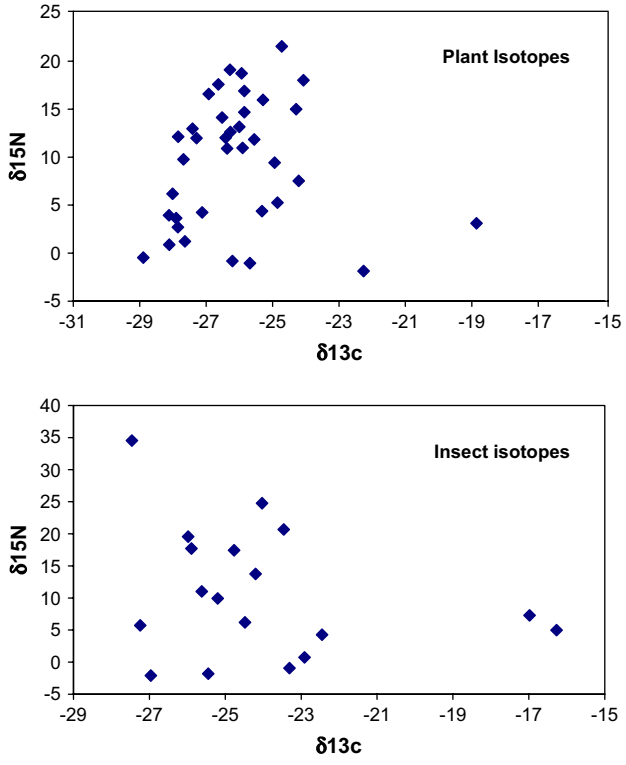

Fig. 1. Stable N and C isotopes for plants and arthropods in Sandia Canyon, August 2001.

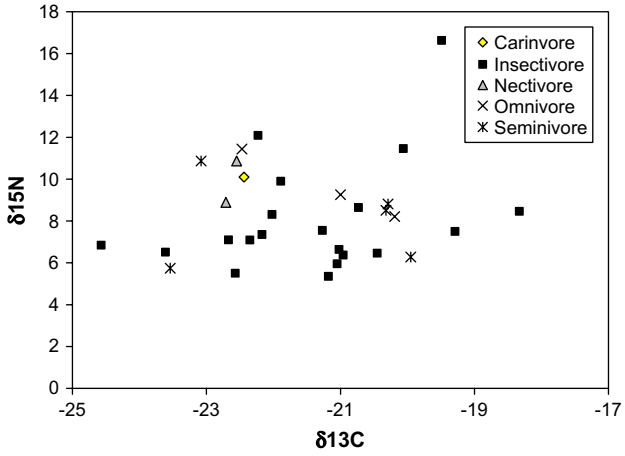

Fig. 2. Stable N and C isotopes for birds in Sandia Canyon, August 2001.

with a diet of C3 plants. For each trophic level, δ^{15}N values were normally distributed (Shapiro–Wilk test). Overall, bird δ^{15}N values (range = 5.5–16.3 δ^{15}N) had a large range and had some elevated values relative to values in North American songbird feathers reported by Hobson (1999) (range means = 3.4–11.6 δ^{15}N). For the δ^{15}N values in feathers there were no differences between trophic groups ($F_{2,30} = 0.39$, $p = 0.68$), although the corrected average sewage content decreased with trophic level increase ($F_{2,30} = 9.08$, $p = 0.0008$).

3.2. Arthropod isotopic signatures

The isotopic signatures for arthropods showed the highest degree of variation with a 98% coefficient of variation for δ^{15}N values relative to the other plants and birds. For δ^{13}C values there was much less variation within arthropods (12%). The highest value analyzed for δ^{15}N in Sandia wetland was a grasshopper instar (*Camnula pellucida*), which had a value of 34.53‰ δ^{15}N indicating that approximately 92% of its N was ultimately derived from sewage N taken up by plants at the base of the food chain.

3.3. Plant isotopic signatures

Plants collected directly in the stream channel had twice as high δ^{15}N on average as plants collected on the wetland edge ($\bar{x}$=13.2channel, SE = 1.3, $\bar{x}$=6.9edge, SE = 1.4) ($F_{1,35} = 10.18$, $p = 0.003$) (refer to Table 3). The N difference in channel and edge plants is more evident in the sewage N ratio with almost a 60% difference in sewage N in the two locations. This was not the case with δ^{13}C in the wetland with no difference in δ^{13}C in the channel and edge of the wetland ($F_{1,35} = 0.01$, $p = 0.97$).

4. Discussion

Sewage nitrate discharge to the wetland increased the δ^{15}N content of plants and insects of the wetland. Channel plants contained 60% more sewage N than plants on the edge of the wetland. This result supports a similar study with

Table 3
Mean stable-carbon and N isotope ratios for plants growing at the upland edge of the wetland or in the channel of Sandia wetland

Species	δ^{13} C	δ^{15}N
Broad-leaved cattail (*Typha latifolia*)		
Edge	−26.5	8.2
Channel	−20.2	14.3
Evening primrose (*Oenothera albicaulis*)		
Edge	−27.8	12.3
Channel	−26.9	16.6
White clover flower (*Trifolium repens*)		
Edge	−28.1	0.9
Channel	−27.9	3.7
Yarrow (*Achillea lanulosa*)		
Edge	−25.3	−0.8
Channel	−26.2	16.0
Wavey thistle (*Cirsium undulatum*)		
Edge	−27.4	12.9
Channel	−27.3	12.0

broad-leaved cattails in Sandia wetlands that found cattails growing near the head of the wetland have δ^{15}N values up to 38‰ suggesting that sewage nitrate is their main source of N with in-situ denitrification leading to further enrichment of the wetland nitrate pool (Heikoop et al., 2002). The results of this study with several different species of plants showed that over a distance as little as 5 m from the main stream, sewage nitrate uptake (and presumably availability) could be reduced in plants by as much 60%. Due to its net negative charge at typical soil pH, dissolved organic matter is generally very mobile through the soil system (Dunnivant et al., 1992). Similarly, Ashworth and Alloway (2003) also found that sewage sludge-derived organic matter in addition to copper and nickel were found to be readily leached through the soil. However, copper and nickel added in the inorganic form lacked mobility through the soil. For Sandia wetland, with the potential for environmental contamination in addition to dissolved organic matter, this could result in increased mobility of contaminants throughout the system. In this study PCBs were not detected in the blood of the birds, suggesting that although nitrogen may be bioavailable, the PCBs might not be available to higher trophic levels due to organic dissolved matter.

Arthropods and plants collected from the wetland had a higher degree of variation in δ^{15}N than we would expect had we been working in a non-effluent fed wetland. Indeed, the arthropod suite contained 98% variability compared to 25% within birds. This may be a result of scale in that the plants obtained nutrients from their immediate vicinity, with high variability in the availability of sewage-derived nitrate, whereas birds obtained food from a larger area (including from outside the wetland), thus decreasing the overall uptake of sewage nitrate. The high variability in arthropods suggests that they are feeding on a variety of plants within the wetland, and that we did not capture the full isotopic variability of plants within the system. This follows the results of other studies where variation in the δ^{15}N signature of primary producers at the base of the food chain can produce variation in δ^{15}N within the primary consumer (e.g. Kline et al., 1993; Kling et al., 1992). Several studies have attempted to remove such variability in order to better identify trophic level differences. These techniques typically rely on comparing secondary or higher consumer δ^{15}N values relative to baseline values of primary consumers, which integrate temporal and spatial variability in primary producer δ^{15}N, thus allowing for a more valid comparison (Vander Zanden et al., 1997; 1999;Post, 2002). The appropriate primary consumer baseline value(s) can be determined based on the δ^{13}C of higher-level consumers (Vaner Zanden, 1999; Post, 2002). This is because there is little trophic fractionation of carbon so δ^{13}C of higher carnivores closely reflects that of herbivores at the base of the food chain. It will be difficult to establish an isotopic baseline in systems like the Sandia wetland, however, given the large isotopic variability in primary producers and consumers over small spatial scales. Indeed, we found no clear trophic level isotopic effects. We can, however, make use of the distinct sewage isotopic signal to track sewage uptake through the food web.

We found no clear trophic level effects between our major groups (plants, arthropods, and birds) or within birds. Our range in δ^{15}N for plants (all primary producers) is equivalent to a trophic level effect equivalent to nearly six trophic levels assuming a fractionation factor of 3.4‰. Obviously, this isotopic range is a function of the varying input of N and the trophic level effects. The range δ^{15}N of insects, which were mostly herbivores, is equivalent to greater than 11 trophic levels. The influx of sewage effluent into a system greatly confounds the ability to predict trophic levels. For example, in this study there was one primarily seed-eating lark sparrow (*Chondestes grammacus*) that had much higher than expected δ^{15}N. Although this species does have dietary changes depending on the location and habitat (Kaspari and Joern, 1993; Ehrlich et al., 1988), we could mistakenly be led to believe, based on isotopic data that it was feeding at an anomalously high trophic level, when in reality its high δ^{15}N was likely driven by the presence of sewage nitrate in the system. Caution is warranted in applying isotopic trophic level techniques in systems with high spatial variability in N inputs of distinct isotopic composition, such as sewage.

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