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Riparian plant isotopes reflect anthropogenic nitrogen perturbations: robust patterns across land use gradients

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Abstract. Riparian plants incorporate nitrogen (N) from aquatic, terrestrial, and atmospheric sources, and their stable isotope compositions ($\delta^{15}N$) may reflect land use impacts on N sources and transformations over scales of sites to watersheds. We surveyed leaf $\delta^{15}N$ values of 11 common riparian tree, shrub, and herbaceous species from 20 streams and rivers spanning three fifth-order watersheds in northern Utah, USA ($n = 255$ sites and 819 leaf samples). Streams spanned undeveloped montane forests to suburban, urban, and agricultural lands. Mean species-specific differences in leaf $\delta^{15}N$ values were relatively small within sites (1.2 ± 2.2‰), although emergent aquatic macrophytes had higher within-site $\delta^{15}N$ values than other growth forms. Leaf $\delta^{15}N$ values varied significantly across land-use categories, and were lowest in undeveloped montane reaches (0.5 ± 1.9‰; mean and standard deviation), intermediate in suburban and urban reaches (2.3 ± 2.6 and 3.2 ± 3.4‰), and greatest in agricultural reaches (4.1 ± 3.1‰). The substantial variation in leaf $\delta^{15}N$ values within a land use category often corresponded with local management differences. In an undeveloped montane canyon permitting off-leash dogs, leaf $\delta^{15}N$ values (1.5 ± 1.3‰) exceeded similar canyons that strictly prohibited dogs ($\delta^{15}N = -0.7 \pm 1.1$‰). Canyons with cattle grazing had leaf $\delta^{15}N$ values enriched by 1.4 and 2.8‰ relative to similar, but un-grazed canyons. Variation in traffic between 0 and 5000 vehicles per day did not significantly affect leaf $\delta^{15}N$ values, although a canyon with 50,000 vehicles per day showed a 5.7‰ increase relative to low-trafficked canyons. Urban leaf $\delta^{15}N$ values were consistently enriched by 2.5 ± 0.6‰ relative to leaves in un-grazed montane reaches, and leaves in a septic-impacted suburban reach were enriched by 4.6‰ relative to upstream samples. Samples from a sewage-impacted urban river averaged 8.0 ± 4.1‰ and reached 22‰ adjacent to publicly owned treatment works (POTW). Another urban river displayed similar values in the absence of POTWs, implicating leaky sewers. Our results demonstrate the capacity of N isotopes from a diverse riparian plant community to inform our spatial understanding of watershed N-cycling perturbations, and illustrate the impact of human activities on N cycling even within protected watersheds.

Key words: agriculture; grazing; isoscape; leaf nitrogen isotope; pet waste; plant species; riparian; septic; sewage; urban; water quality.

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Introduction

Waterways in human-impacted ecosystems receive nitrogen (N) from numerous point and diffuse sources that contribute to eutrophication and alter the composition and function of riparian and aquatic communities. Teasing apart the spatial variation of potential N sources is difficult in heterogeneous modern landscapes, which are often characterized by patchworks of natural vegetation, suburban, urban, and agricultural lands. Fertilizer, atmospheric deposition, animal waste, waste water effluent, and septic systems typically represent important N sources in urban, urbanizing, and agricultural watersheds (Baker et al. 2001, Groffman et al. 2004, Lewis and Grimm 2007, Bernhardt et al. 2008, Diebel and van der Zanden 2009, Kaushal et al. 2011, Hale et al. 2014). Determining the spatial distribution of human perturbations to N cycling represents an important prerequisite for developing effective watershed nutrient management strategies. High-resolution spatial sampling of nitrogen stable isotope ratios ($\delta^{15}N$) of riparian plants could provide a useful index for assessing impacts of land use and management on terrestrial and aquatic nitrogen sources and cycling over multiple spatial scales, ranging from individual point sources to stream reaches to entire watersheds.

Spatial variation in $\delta^{15}N$ values reflects differences in ecosystem N sources as well as subsequent fractionations during N cycling. Nitrogen sources from bacterial fixation and inorganic fertilizer typically have $\delta^{15}N$ values close to 0‰, human and animal waste typically have much greater $\delta^{15}N$ values (4–25‰), and atmospheric reactive N varies greatly in isotopic composition depending on source contributions (Aravena et al. 1993, Anisfeld et al. 2007, Bateman and Kelly 2007, Kendall et al. 2007). Subsequent gaseous N losses such as denitrification and ammonia (NH_3) volatilization typically enrich the $\delta^{15}N$ values of remaining N. As a consequence, land use types associated with increased anthropogenic N inputs (agricultural, suburban and urban) often show greater $\delta^{15}N$ values than natural landscapes (Peipoch et al. 2012). This pattern is especially evident in aquatic ecosystems that integrate large watersheds, where $\delta^{15}N$ values often scale with total N loads, human population, and the sum of urban and agricultural land cover (Cabana and Rasmussen 1996, Mayer et al. 2002, Anderson and Cabana 2006, Kohzu et al. 2008).

Analysis of N isotopes in biological tissues provides an effective method of temporal integration, as tissue $\delta^{15}N$ values reflect the mass-weighted isotope ratio of N sources after accounting for any assimilatory or biochemical fractionation (Costanzo et al. 2001, Evans 2001). Previous studies have used N isotope ratios in aquatic organisms and vascular plants to infer spatial patterns in N sources and fluxes at scales from sites (m) to watersheds (km; Harrington et al. 1998, Costanzo et al. 2001, Hebert and Wassenaar 2001, Steffy and Kilham 2004, Anderson and Cabana 2006, Diebel and van der Zanden 2009, Atkinson et al. 2013). Aquatic macrophytes have been used as N isotope bioindicators (Cole et al. 2005, Kohzu et al. 2008, Peipoch et al. 2012, Pastor et al. 2013), yet riparian trees and shrubs have seldom been used in this fashion. Trees and shrubs often dominate riparian vegetation, and similar species often span broad climate gradients. Also, riparian plants often thrive in heavily disturbed urban and agricultural waterways where other taxa can be difficult to consistently encounter. Finally, riparian plants integrate both terrestrial and aquatic N sources. Streams and riparian groundwater, in addition to soils, can provide major N sources to riparian vegetation (Helfield and Naiman 2001, Schade et al. 2005, Hultine et al. 2008, Lisuzzo et al. 2008). Plants can also potentially assimilate reactive N directly from the atmosphere, which can represent a significant source in proximity to roads or industry (Ammann et al. 1999, Stewart et al. 2002).

Here, we measured leaf $\delta^{15}N$ values in riparian plants to assess potential impacts on N sources and cycling associated with a broad spectrum of land-use and management attributes across northern Utah watersheds. To ascertain whether differences in plant stress or water availability across these land-use gradients might also contribute to variation in plant N uptake or source differences (e.g., soils vs. groundwater), we analyzed leaf C isotope ($\delta^{13}C$) values. Leaf $\delta^{13}C$ values in C3 plants reflect the difference between atmospheric and leaf intercellular carbon dioxide (CO_2) concentrations (Farquhar et al.

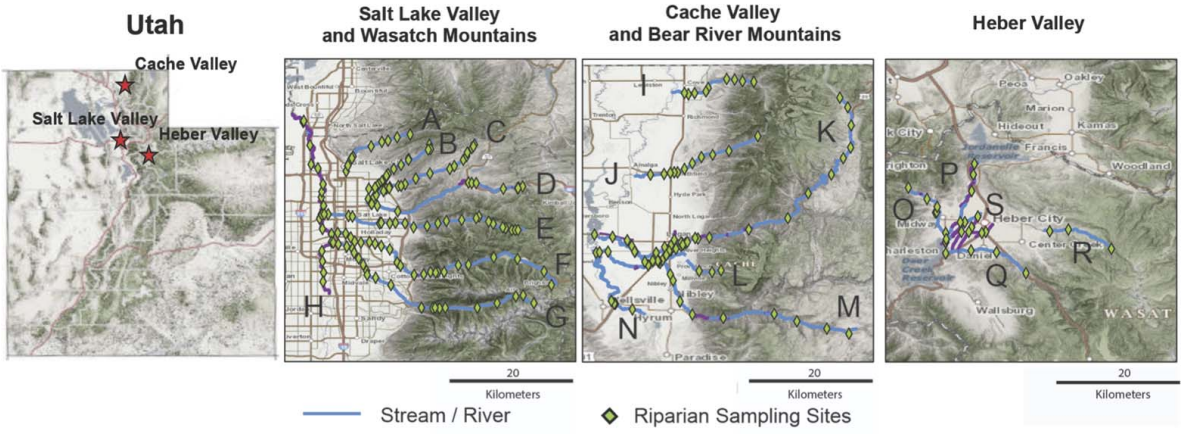

Fig. 1. Streams and vegetation sampling sites in the Salt Lake, Cache, and Heber Valleys, USA. Streams and rivers are indicated with lines and sampling sites are denoted by diamonds. Letters indicate stream names. In the Salt Lake Valley: City Creek (A), Red Butte Creek (B), Emigration Creek (C), Parley's Creek (D), Mill Creek (E), Big Cottonwood Creek (F), Little Cottonwood Creek (G), Jordan River (H). In the Cache Valley: High Creek (I), Summit Creek (J), Logan River (K), Spring Creek (L), Blacksmith Fork Creek (M), and the Little Bear River (N). In the Heber Valley, Snake Creek (O), Provo River (P), Daniel's Creek (Q), Rock Creek (R), and Lake Creek (S).

1989). This balance reflects factors controlling CO_2 supply via stomatal conductance (e.g., water stress), as well as factors controlling CO_2 demand at the leaf level (e.g., light and enzyme activity). Previous work has also identified subtle plant species-specific differences in leaf N isotope composition (Pardo et al. 2006, Kahmen et al. 2008) that could complicate the use of diverse riparian plant communities to infer spatial patterns in ecosystem $\delta^{15}N$ values. Accordingly, we first assessed the magnitude of species-specific differences in leaf $\delta^{15}N$ values within sites, as well as patterns in species distribution across the landscape. This allowed us to evaluate and remove biased species when assessing landscape-scale patterns. Then, we assessed relationships between land-use typologies and local management features and leaf $\delta^{15}N$ values, and explored possible mechanisms underlying these patterns.

Methods

Site descriptions

We sampled common riparian plant species along streams and rivers that drained into three major montane valleys in northern Utah, USA: the Salt Lake Valley, the Cache Valley, and the Heber Valley (Fig. 1). These watersheds represent transitions from largely undeveloped montane forests (containing national forest lands and federal wilderness areas) to valley bottoms with urban, suburban, and/or agricultural land uses. The highly urbanized Salt Lake Valley has a population exceeding one million, whereas the nearby Cache and Heber Valleys are predominantly agricultural, with human populations of approximately 112,000 and 26,000, respectively. We sampled multiple streams within each watershed (total $n = 20$; Fig. 1; Appendix: Table A1). Attributes such as the presence of homes with septic systems were determined from public documents. Most of the montane streams paralleled roads varying in traffic density from <5 to ~50,000 cars per day (Appendix: Table A1). Sampling sites were typically located 5–20 m from roads.

In the Salt Lake Valley, the seven sampled streams flowed from montane canyons with differing land-use regulations into urban Salt Lake City and adjacent municipalities. These streams terminate at the Jordan River (Fig. 1), which flows through the center of the urbanized Salt Lake Valley. In the Cache Valley, streams descend from montane headwaters and traverse suburban, urban and/or agricultural lands before joining the Little Bear River and associated wetlands. Streams in the Heber Valley primarily

Table 1. Characteristics of sampled riparian plant species.

Species	Abbreviation	Growth form	Position	Potential mycorrhizal association	Land use preference
Acer grandidentatum	ACGR	tree	riparian/upland	AM	montane, suburban
Acer negundo	ACNE	tree	riparian/upland	AM	...
Betula occidentalis	BEOC	tree	riparian	ECM	montane, suburban
Cornus stolonifera	COST	shrub	riparian	AM	agricultural, montane
Phalaris arundinacea	PHAR	herbaceous	riparian/emergent aquatic	AM	agricultural, suburban, urban
Phragmites australis	PHAU	herbaceous	riparian/emergent aquatic	AM	urban
Populus fremontii	POFR	tree	riparian/upland	ECM, AM	agricultural, suburban, urban
Populus angustifolia	POAN	tree/shrub	riparian/upland	ECM, AM	...
Salix exigua	SAEX	shrub	riparian	ECM, AM	...
Typha latifolia	TYLA	herbaceous	riparian/emergent aquatic	AM	agricultural
Ulmus pumila	ULPU	tree	riparian/upland	AM	agricultural, suburban, urban

Notes: Growth form denotes whether the taxon is a tree, shrub, or herbaceous plant, and position reflects the typical topographic distribution of the taxon relative to the stream channel. Potential associations with two primary forms of mycorrhizae (arbuscular and ecto) are denoted AM and ECM, respectively (Lodge 1989, Brundrett 2009). Significant land use preferences (agricultural, montane, suburban, urban) were assessed using indicator species analysis; ellipses denote the absence of significant land use preferences.

flow through suburban and agricultural landscapes. The Provo River is the principal waterway of the Heber Valley, flowing from the Jordanelle Reservoir through a mixed suburban, low-density urban, and agricultural landscape.

We sampled riparian vegetation at multiple sites on each stream to capture variation in $\delta^{15}N$ values associated with land use (total $n = 255$ sites). Microbial N fixation is associated with some riparian species such as Russian olive (*Elaeagnus angustifolia*), an invasive riparian tree prevalent throughout the southwestern USA. Plants associated with N-fixing bacteria (and sites where they dominated) were excluded from this study. Sampling intensity differed among streams according to stream length, land use variation, and access constraints (Fig. 1). Sites consisted of a ~30 m transect parallel to the stream, including both sides of the stream when possible. We sampled leaves from up to 11 species representative of the local flora (Table 1) when present. Mean richness of target species measured 3.1 ± 1.3 (standard deviation), representing a total of 819 samples. Plants were only sampled if they were in close proximity (<2 m) of the perennial channel or were visibly rooted in the stream. At each site, we collected 5–15 mature sun and shade leaves from each species. Where multiple individuals of the same species were present at the same site, we sampled leaves from up to five individuals and composited by species. We sampled most Salt Lake Valley sites during late September/early October 2013, and Cache and Heber Valley sites in late July 2014. We sampled additional montane Salt Lake Valley sites in July 2014 and resampled a subset of 2013 sites. Temporal changes in leaf $\delta^{15}N$ values were not significant, in agreement with other studies (Kohzu et al. 2008, Pastor et al. 2014), and thus we pooled the data from the two collection periods. Leaves were oven-dried at 70°C, ground, and analyzed for N and C isotope ratios and elemental content at the Stable Isotope Ratio Facility for Environmental Research at the University of Utah (http://sirfer.utah.edu). We report N and C isotope ratios in δ notation relative to atmospheric N_2 and VPDB, respectively, with a mean analytical precision <0.2‰.

Statistical analysis

We assessed species-specific differences in N isotope composition (prior to pooling data from different species) by calculating the difference in leaf $\delta^{15}N$ values for each species relative to two abundant and broadly distributed species (*Populus angustifolia* and *Acer negundo*, hereafter termed reference species) when they co-occurred at the same site. Next, we assessed spatial biases in species distribution among land-use categories (urban, agricultural, suburban, and montane) using indicator species analysis in the R package indicspecies (Cáceres and Legendre 2009). Species with large biases in $\delta^{15}N$ values (>1.5‰ relative to reference taxa) and spatial distribution were excluded from subsequent landscape-scale analyses. Based on these criteria, only two of the

eleven species were excluded from subsequent analyses, the emergent aquatic macrophytes *Phragmites australis* and *Typha latifolia*.

We assessed relationships between land use and $\delta^{15}N$ values within and among streams and watersheds using mixed effects models that included sites as random effects to account for intra-site correlations. Models were fit for each stream, each watershed, and the entire dataset. The latter two model categories also included random effects for each stream to account for spatial co-variation. Land use classifications (montane, urban, suburban, agricultural) were determined using aerial imagery and ground truthing, and were included in models as fixed effects. "Montane" sites were located in natural montane vegetation that was undeveloped with the exception of occasional scattered cabins. Sites with low-density residential development outside of incorporated cities were classified as "suburban." "Urban" sites were located within urban boundaries or were downslope of urban development. Sites adjacent to irrigated cropland or irrigated pasture were classified as "agricultural." All statistical models were fit with the lme function in R (Pinheiro et al. 2015), and pairwise differences assessed using the Tukey comparison. We report means and standard deviations as opposed to standard errors given the differences in sample size among streams and land use categories.

Trends in leaf $\delta^{15}N$ values with distance along streams were often non-linear, so we assessed trends with distance for each stream using generalized additive models (GAM) with a Gaussian link function, fitted using the mgcv package in R (Wood 2006). Briefly, GAMs fit smooth trends to data using regression splines that are penalized according to their "wiggliness" during model fitting, thus achieving a statistically optimum degree of curvature. The extent of model curvature can be expressed in terms of degrees of freedom (df) analogous to the order of a polynomial, but allowing for fractional df. Here, we averaged leaf $\delta^{15}N$ values at each site and analyzed each stream separately, including a smooth function of distance downstream from the upper-most site in each model. We assessed the significance of smooth functions of distance as predictors of leaf $\delta^{15}N$ values using *F* tests, and where significant, we reported the effective degrees of freedom of the smooth terms.

Relationships between leaf $\delta^{15}N$ values and continuous predictors

For each sampling location, we calculated elevation, the distance to the nearest road (public communication, State of Utah; http://gis.utah.gov/data/sgid-transportation/roads- system) and the distance to the nearest road with a speed limit $\geq$65 miles per hour using ArcGIS 10.2. We also calculated the traffic density of the nearest road and of all roads within 0.1, 0.5, and 1 km of each sampling location using the annual average traffic density dataset from the Utah Department of Transportation (http://gis.utah.gov/data/sgid-transportation/roads-system/). Within the same spatial buffers, we calculated the average imperviousness using 30-m data from the National Land Cover Database (Jin et al. 2013). Relationships between leaf $\delta^{15}N$ values and these predictors were assessed graphically and using linear regression.

Results

Species-specific variation in $\delta^{15}N$ and $\delta^{13}C$ values and land use fidelity

Plant species from the same sites often varied significantly in leaf $\delta^{15}N$ values relative to co-occurring individuals of the two reference trees *P. angustifolia* and *A. negundo* (Fig. 2). The emergent aquatic macrophytes *Phragmites australis* and *Typha latifolia* showed the greatest biases; both species had mean differences in leaf $\delta^{15}N$ values $> 2‰$ relative to the reference species. Excluding these two taxa, mean species-specific biases relative to *P. angustifolia* and *A. negundo* were relatively small relative to landscape-scale spatial patterns (shown later). Absolute values of mean species differences relative to *P. angustifolia* and *A. negundo* varied from 0.2 to 2.8‰ for the eight remaining species, with means and standard deviations of 1.2 ± 2.2 and $1.1 \pm 2.3‰$. The average standard deviation of leaf $\delta^{15}N$ values within a given site measured 1.6‰. However, 60 of the 255 sites had standard deviations >2‰, indicating the importance of within-site variability in the isotope composition of N sources in addition to plant species-specific effects.

Co-occurring species showed relatively low variability in leaf $\delta^{13}C$ values. Mean species-

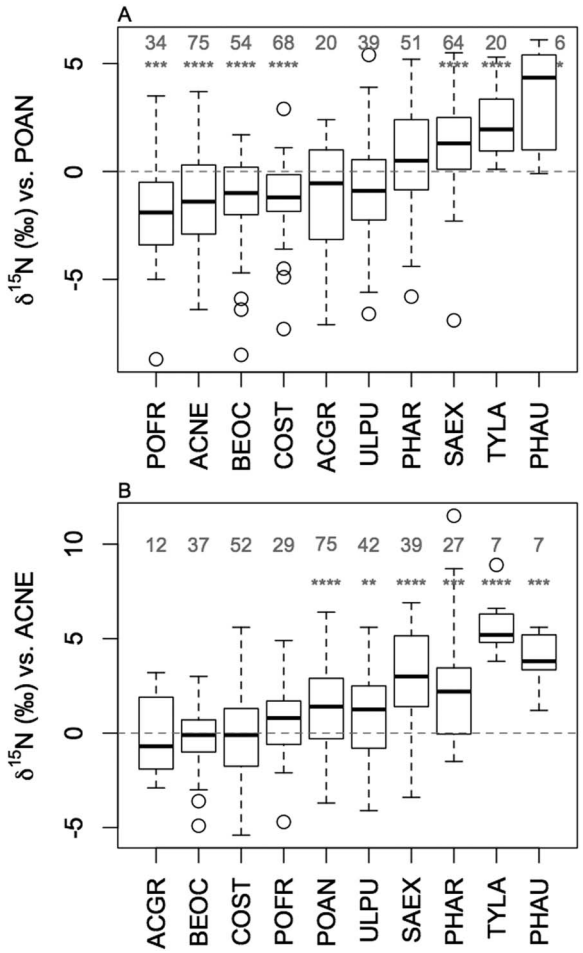

Fig. 2. Intra-site differences in leaf $\delta^{15}N$ for each sampled species relative to (A) *P. angustifolia* (POAN), and (B) *A. negundo* (ACNE). Boxes represent medians and the interquartile range, and circles represent data > 1.5 times the range from the box to the whiskers. Four-letter species abbreviations are given in Table 1. Sample size for each comparison is shown in grey text in the upper portion of each panel. Statistical significance (difference from zero) is reflected by asterisks, representing $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$, respectively.

specific differences in $\delta^{13}C$ relative to *P. angustifolia* and *A. negundo* measured 0.6 ± 0.9 and 0.4 ± 0.7‰, respectively. Similarly to $\delta^{15}N$, several of the species-specific differences in $\delta^{13}C$ values were statistically significant due to the high statistical power afforded by our sampling design, but were of small magnitude (Appendix: Fig. A1). Overall, mean species-specific $\delta^{13}C$ values varied between −30.4 and −28.4‰ (Appendix: Fig. A1).

Several species showed significant land use preferences according to indicator species analysis (Table 1), although only two species showed significant fidelity to a single land use category (*P. australis* for urban reaches, and *T. latifolia* for agricultural reaches). Because of their restricted land-use distributions and their relatively large mean isotopic differences relative to other co-occurring taxa (2.3–5.7‰), we excluded *T. latifolia* and *P. australis* from subsequent spatial comparisons. The distribution of other species either showed no relationship to land use (*A. negundo*, *P. angustifolia*, *S. exigua*), or species were associated with multiple land use types (Table 1; *A. grandidentatum*, *B. occidentalis*, *C. stolonifera*, *P. arundinacea*, *P. fremontii*, *U. pumila*). Species distribution with elevation typically coincided with land use preferences (Appendix: Fig. A2). *Phragmites australis*, *P. arundinacea*, *P. fremontii*, and *U. pumila* were predominantly encountered on the valley bottoms, whereas *B. occidentalis* and *A. grandidentatum* were typically encountered at higher elevations. However, several of the most common species (*P. angustifolia*, *C. stolonifera*, *S. exigua*, *A. negundo*) spanned a wide elevation range, including valley bottom and montane sites, allowing robust comparisons of leaf $\delta^{15}N$ values across land use types while controlling for species identity.

Variation in $\delta^{15}N$, C/N, and $\delta^{13}C$ values within and among land use categories

Leaf $\delta^{15}N$ values varied from −4.4 to +22.6‰ among samples and significantly differed by land use category ($p < 0.0001$). Values were lowest in the montane stream reaches (mean and standard deviation of 0.5 ± 1.9‰), intermediate in the suburban and urban reaches (2.3 ± 2.6 and 3.2 ± 3.4‰, respectively), and greatest in the agricultural reaches (4.1 ± 3.1‰; Fig. 3). Notably, individual samples from each land use category differed by more than 10‰, indicating the importance of local factors in addition to the broad land-use typologies in influencing isotope composition. Stream-level differences in leaf $\delta^{15}N$ values within the broader montane, suburban, urban, and agricultural typologies were also apparent (Fig. 4) and are described below. Comparisons among stream reaches were similar

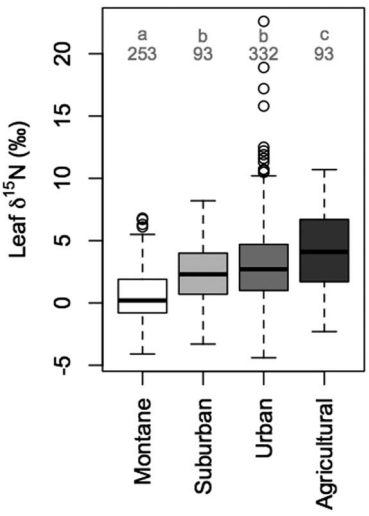

Fig. 3. Boxplots of leaf $\delta^{15}N$ by land use categories with sample size in grey text; letters denote significant differences assessed by Tukey comparisons in a mixed-effects model ($P < 0.001$ for all comparisons).

when based on raw leaf $\delta^{15}N$ values or leaf $\delta^{15}N$ values normalized to *P. angustifolia* (data not shown). Thus, we focused our subsequent analysis on the raw data for ease of interpretation.

Leaf C/N mass ratios also varied according to land use ($p < 0.0001$). Montane leaf samples had significantly greater C/N (25 ± 8; mean and standard deviation) than the urban (23 ± 7) or agricultural (20 ± 6) samples, while the suburban samples were intermediate (23 ± 10). Despite these strong landscape-scale differences in C/N ratios across land use categories, C/N ratios did not differ significantly among land use categories at the level of individual streams due to high variability.

In contrast to the $\delta^{15}N$ values and C/N ratios, leaf $\delta^{13}C$ values varied little among land use types, although agricultural reaches had slightly but significantly greater values (−29.1 ± 1.4‰; mean and standard deviation) than montane (−29.8 ± 1.5‰) or urban reaches (−30.1 ± 1.6‰). At the scale of individual streams, only three streams showed significant differences in leaf $\delta^{13}C$ values between land use categories (Parley's Creek, Daniel's Creek, and Lake Creek; Appendix: Fig. 3).

Patterns in urban and suburban leaf $\delta^{15}N$ values

In the Salt Lake Valley, mean leaf $\delta^{15}N$ values in six separate urban stream reaches were 2.5‰ greater and significantly different ($p < 0.05$ for the land use main effect in each mixed effects model) than each of their corresponding montane reaches. In the Cache Valley, Summit Creek showed a similar increase of 2.4‰ ($p < 0.05$) from montane to urban sites. However, the Logan River and Blacksmith Fork Creek had relatively high leaf $\delta^{15}N$ values (1.4 and 2.8‰) in the montane reaches that did not significantly differ in the downstream urban and agricultural reaches. In the Heber Valley, montane to urban transitions could not be sampled on the same waterways, although samples from urban ditches were 5.7‰ greater on average than nearby montane samples.

Many of the plants along urban stream reaches from all three valleys had similar leaf $\delta^{15}N$ values (2.5 ± 1.6‰, $n = 11$ streams) despite large differences in urban form and land-use characteristics. However, the Jordan River in the Salt Lake Valley (which receives inputs from all of the other Salt Lake Valley streams) had leaf $\delta^{15}N$ values (8.0 ± 4.1‰) that were significantly greater ($p < 0.0001$) than the other urban waterways in the Salt Lake and Cache Valleys (Fig. 4). Urban ditches in the Heber Valley had the second highest leaf $\delta^{15}N$ values (6.1 ± 1.0‰), which did not significantly differ from the Jordan River. Plants along the other urban streams in the Salt Lake Valley (Fig. 4) had statistically similar leaf $\delta^{15}N$ values when Mill Creek sites potentially influenced by POTW effluent were excluded. There was no apparent relationship between the presence of heavily fertilized golf courses adjacent to Emigration Creek and Parley's Creek and leaf $\delta^{15}N$ values at adjacent or downstream sites.

As with urbanization, the presence of scattered suburban development was similarly associated with increased plant $\delta^{15}N$ values in streams from all three valleys when compared with upstream montane reaches. These streams included Emigration Creek, High Creek, and Snake Creek (Fig. 4). Suburban stream reaches generally had statistically similar leaf $\delta^{15}N$ values (2.2 ± 1.5‰), although plants in Emigration Creek were significantly greater (3.9 ± 2.9‰) than those from either Summit Creek or High Creek (−0.6 ± 1.7 and 0.7 ± 1.8‰, respectively). Homes in the

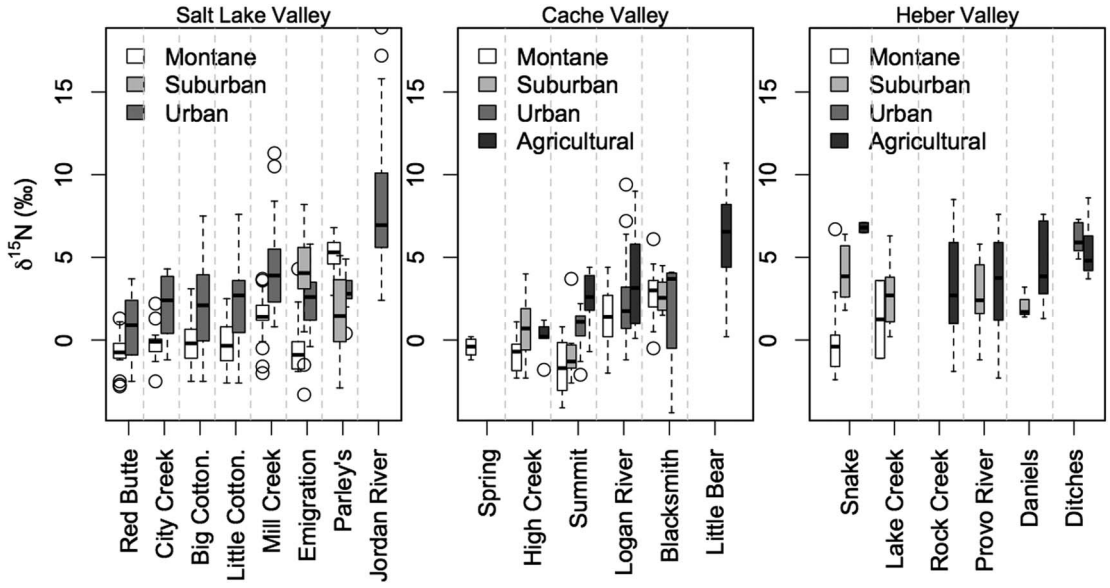

Fig. 4. Boxplots of leaf $\delta^{15}N$ values by stream and land use categories for the Salt Lake, Cache, and Heber Valleys. Two samples with $\delta^{15}N$ values > 20‰ in the Jordan River were omitted for clarity.

Emigration Creek watershed rely on septic waste disposal, and leaf $\delta^{15}N$ values in this suburban reach were greater by 4.2‰ relative to upstream montane samples.

Patterns in agricultural leaf $\delta^{15}N$ values

We found little variability in leaf $\delta^{15}N$ values among agricultural streams in the Heber Valley, and very high variation (6‰) in mean $\delta^{15}N$ values among agricultural streams in the Cache Valley (Fig. 4). Plants along High Creek had the lowest leaf $\delta^{15}N$ values of all of the agricultural reaches (0.1 ± 1.2‰), whereas leaf $\delta^{15}N$ values along the nearby Little Bear River were significantly greater ($p < 0.001$) at 6.1 ± 2.7‰, and leaf $\delta^{15}N$ values in the Logan River and Summit Creek were intermediate (Fig. 3).

Patterns in montane leaf $\delta^{15}N$ values

Montane reaches in all three watersheds displayed large differences in leaf $\delta^{15}N$ values coinciding with local land use variation. In the Salt Lake Valley, five of the six montane stream reaches (City Creek, Emigration Creek, Red Butte Creek, Little Cottonwood and Big Cottonwood Creek) had similarly low leaf $\delta^{15}N$ values (−0.4 ± 0.2‰; Fig. 4) regardless of differences in traffic volume, which varied from <5 to 5000 vehicles per day. However, samples from Parley's Creek adjacent to Interstate Highway 80 (with approximately 50,000 vehicles per day) had significantly greater leaf $\delta^{15}N$ values (5.1 ± 1.3‰, $p < 0.0001$) than the other montane reaches. The upper reach of Parley's Creek is also several hundred meters downstream of Summit Park, a community reliant on septic waste disposal. Leaf $\delta^{15}N$ values along Mill Creek (1.5 ± 1.3‰), the only montane reach permitting off-leash dogs, were also significantly greater ($p < 0.0001$) than the other montane canyons (with the exception of Parley's Creek). Mill Creek also contains a heavily used youth camp at the bottom of the montane reach, although no significant isotope trends with downstream distance were evident (Fig. 5). Plants in protected montane reaches in the Cache and Heber Valleys had similar $\delta^{15}N$ values to those in the Salt Lake Valley. However, the two Cache Valley montane reaches characterized by wildland cattle grazing, or grazing combined with concentrated elk feeding (Appendix: Table A1), had significantly greater $\delta^{15}N$ values than their counterparts that lacked intensive ungulate grazing ($p < 0.0001$; 1.4 and 2.8‰ for the Logan River and Blacksmith Fork Creek, respectively).

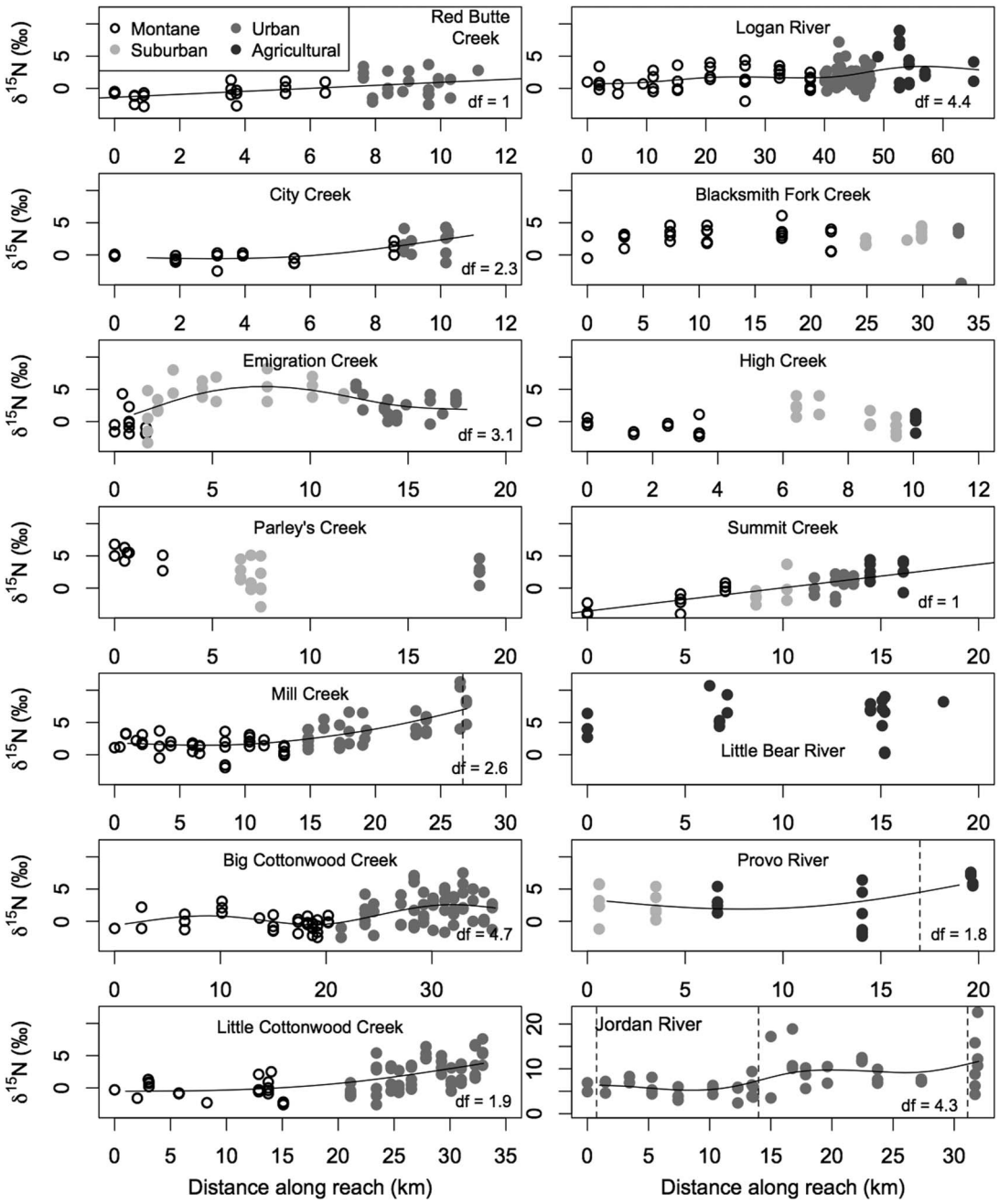

Fig. 5. Trends in leaf $\delta^{15}N$ values with downstream distance along each stream plotted by land use category. Note that y-axes are similar among plots except for the Jordan River (bottom right), and x-axes differ. Trends with distance were assessed using additive models and fitted smooth functions are shown when significant ($p < 0.05$), with estimated degrees of freedom (df) of the smooth trend shown in the bottom right of each panel. Vertical dashed lines denote the approximate location of POTW inputs.

Trends in leaf $\delta^{15}N$ values with stream distance

Patterns in leaf $\delta^{15}N$ values with downstream distance varied among streams and land-use categories (Fig. 5). In the Salt Lake Valley, leaf $\delta^{15}N$ values often did not vary with distance along montane reaches and then increased approximately linearly with distance in urban reaches. This pattern was illustrated in plants sampled along City Creek, Mill Creek, and Little Cottonwood Creek. Big Cottonwood Creek also fit this pattern, excepting a significant spike in leaf $\delta^{15}N$ values (as reflected by the GAM smooth function) immediately downstream of a campground. In contrast, leaf $\delta^{15}N$ values along Red Butte Creek showed a significant linear increase across the entire montane to urban gradient. In Emigration Creek, leaf $\delta^{15}N$ values spiked at the interface between montane sites and suburban homes with septic systems, remained elevated throughout the suburban montane reach, and subsequently decreased along the urban reach.

In the Cache and Heber Valleys, leaf $\delta^{15}N$ values generally showed fewer coherent trends with distance along stream reaches. Summit Creek values significantly increased along the montane to urban gradient, and the Logan River showed an oscillating but overall increasing trend with downstream distance. Leaf $\delta^{15}N$ values along Blacksmith Fork Creek, High Creek, and the Little Bear River displayed no significant trend with distance, while leaf $\delta^{15}N$ values along the Provo River showed a weak parabolic trend driven by a site downstream of a POTW plant.

Leaf $\delta^{15}N$ values displayed a variable relationship to the presence of POTW plants, which were located upstream and throughout the sampled portion of the Jordan River (Fig. 5). The Jordan Basin Water Reclamation Facility was upstream of our first riparian sampling site. Samples from sites that bracketed the South Valley Water Reclamation facility did not differ in $\delta^{15}N$ values (6.3 ± 1.4 vs. 5.9 ± 1.4‰; Fig. 5), and downstream samples remained similar (5.6 ± 1.8‰) until after the confluence with Mill Creek, which receives effluent from the Central Valley Water Reclamation Facility. Mill Creek leaf samples adjacent to these POTW inputs were enriched (7.8 ± 3.0‰) relative to upstream urban samples from Mill Creek sites (3.5 ± 1.8‰). Similarly, Jordan River samples had highly variable and enriched leaf $\delta^{15}N$ values (12.0 ± 6.2‰) downstream of the Central Valley POTW inputs within a distance of approximately 3 km. Leaf $\delta^{15}N$ values ultimately decreased (8.9 ± 2.2‰) but remained elevated relative to samples upstream of the Central Valley POTW inputs. Approximately 17 km downstream, samples immediately adjacent to the Salt Lake City Water Reclamation plant again showed a sharp increase in $\delta^{15}N$ values (15.2 ± 6.5‰).

Relationships between leaf $\delta^{15}N$ values and proxies for climate, traffic, and urbanization

Across the entire dataset, leaf $\delta^{15}N$ values decreased with elevation due to co-variation in urban and montane land use, but they did not significantly vary with elevation after accounting for land use. Leaf $\delta^{15}N$ values showed very weak positive ($R^2 < 0.12$) but statistically significant ($p < 0.0001$) relationships with traffic density and impervious area, and a negative relationship with distance to the nearest road when considering the dataset as a whole. These relationships became insignificant when assessed separately within the urban or montane land use categories.

Discussion

We evaluated anthropogenic perturbations to N cycling across watersheds encompassing diverse land use gradients in northern Utah, USA, using measurements of the leaf N isotope composition of a mixed assemblage of common riparian plant species. Although we often observed substantial variation in leaf $\delta^{15}N$ values within a site, systematic species-specific biases were relatively small. Thus, leaf $\delta^{15}N$ values could provide a coherent signal of land use impacts on N sources/cycling when multiple samples were averaged over different spatial scales of interest. These included individual sites adjacent to landscape features, or stream reaches with similar land use characteristics. Leaf $\delta^{15}N$ values provided a particularly sensitive metric for comparing undeveloped montane stream reaches under distinct management regimes. In wildland and suburban areas, we detected the impact of both dogs and cattle on leaf $\delta^{15}N$ values along stream reaches where each roam freely, as well as human waste inputs from suburban development and campgrounds with vaults that are likely leaking.

Overall, we observed a strong and consistent enrichment of leaf $\delta^{15}N$ values in many of the urban and agricultural streams relative to undeveloped montane reaches, consistent with previous work in both aquatic and terrestrial ecosystems (Cabana and Rasmussen 1996, Mayer et al. 2002, Kohzu et al. 2008, Wang and Pataki 2010, Peipoch et al. 2012). However, we also found significant variation in leaf $\delta^{15}N$ values within and among urban streams indicative of heterogeneity in urban N sources and cycling. The variability of N isotopes *along* urban streams and the potential underlying mechanisms have received less attention in previous analyses of land use patterns in N isotopes (Peipoch et al. 2012). Many of the plants along urban stream reaches showed increasing leaf $\delta^{15}N$ values with distance downstream. These trends implied increased contributions of isotopically-enriched N with increasing watershed area, and/or a cumulative increase in fractionating gaseous losses.

Species-specific variation in leaf $\delta^{15}N$ values

Global meta-analyses have often used leaf $\delta^{15}N$ values as a proxy for the N status of natural ecosystems, where enrichment in $\delta^{15}N$ values is thought to reflect a relative increase in N availability and the prevalence of denitrification relative to leaching (Craine et al. 2009*b*, Houlton and Bai 2009). Abiotic (e.g., climate) and biotic factors (e.g., form of mycorrhizal association) appear to show strong relationships with leaf $\delta^{15}N$ values over broad spatial scales irrespective of plant species composition (Craine et al. 2009*b*). Yet, fewer studies have quantitatively addressed the potential impacts of species-specific variation on the use of N isotopes to assess N-cycling processes (e.g., Hobbie et al. 2000, Pardo et al. 2006, Kahmen et al. 2008). For example, as a consequence of internal biochemical fractionations, leaf $\delta^{15}N$ values can potentially vary among species even when they are using the same N sources (Evans 2001). Our data allowed assessment of species-specific differences with high statistical power, which was necessary given the high potential variability in leaf $\delta^{15}N$ values among species from the same sites. Our findings suggest that with some caveats, systematic variation in N isotopes among species was suitably small that most species could be pooled to assess landscape-scale patterns in $\delta^{15}N$ values. This finding is important in that larger-scale analyses have often pooled $\delta^{15}N$ values from disparate species without assessing potential impacts of species-specific variation.

However, biases in leaf $\delta^{15}N$ values were highly significant among several of the species sampled. Kahmen et al. (2008) found that some inter-specific variation in leaf $\delta^{15}N$ values among grassland species could be explained by N source preferences (ammonium vs. nitrate). In our study, spatial heterogeneity in N sources, hydrologic flow paths, and species differences in rooting depth and N contributions from stream vs. soil water likely also contributed to isotopic differences. Nevertheless, broad similarities in leaf $\delta^{13}C$ values among species and across land use gradients implied that samples had similar leaf to air CO_2 gradients. These patterns suggest that differences in plant water availability were minimal among these species and sites, and that these riparian plant species likely thus had similar access to stream N in addition to soil N (Helfield and Naiman 2001, Schade et al. 2005, Hultine et al. 2008, Lisuzzo et al. 2008).

All of the genera sampled in our study are capable of forming mycorrhizal associations, which might also have contributed to species-specific differences in leaf $\delta^{15}N$ values. Here, species from two genera that have been documented to form ectomycorrhizal associations, *P. angustifolia* and *S. exigua* (Lodge 1989), showed small but significant increases in mean leaf $\delta^{15}N$ values relative to an arbuscular reference species, *A. negundo*. Pardo et al. (2006) found a similar increase in leaf $\delta^{15}N$ in ectomycorrhizal trees relative to co-occurring arbuscular trees in the northeastern USA. However, these patterns are opposite those observed in a global meta-analysis, where ectomycorrhizal plant species were slightly depleted in $\delta^{15}N$ (1.2‰) relative to arbuscular plants (Craine et al. 2009*b*). Mechanisms by which mycorrhizae impact leaf $\delta^{15}N$ values remain under debate, as empirical patterns and controlled experiments have yielded conflicting results (Evans 2001).

We observed the greatest species-specific differences in leaf $\delta^{15}N$ values in the emergent aquatic species *T. latifolia* and *P. australis*. The fact that these taxa were rooted directly in the perennial channel as opposed to the stream bank

implies that any NO_3^- assimilated by their roots may have been subject to isotopic enrichment from benthic denitrification, thus potentially increasing their leaf $\delta^{15}N$ values relative to other species that had incompletely submerged roots or had root mats floating in the channel itself (e.g., *P. angustifolia* and *A. negundo*). A similar increase in leaf $\delta^{15}N$ values in *P. australis* relative to co-occurring riparian species was observed in Japanese streams and attributed to localized denitrification (Kohzu et al. 2008). The grass *Phalaris arundinacea* and the wetland shrub *S. exigua* were also frequently rooted in stream sediments, and were significantly enriched relative to our reference taxa, but to a lesser degree than *T. latifolia* and *P. australis*. The former species tend to establish at a broader range of topographic positions, and variation in their leaf $\delta^{15}N$ values across stream channel cross-sections could potentially provide a metric for assessing spatial variation in denitrification.

Interpreting increases in leaf $\delta^{15}N$ values along urban stream reaches

We observed two general patterns in leaf $\delta^{15}N$ values in the urban stream reaches: high and variable values in reaches receiving effluent from POTW plants, and a smaller but consistent 2–3‰ increase in many of the other urban reaches relative to their montane counterparts. The importance of POTW plants in providing a large source of isotopically-enriched N in urban waterways is well known (Sebilo et al. 2006, Kendall et al. 2007), yet potential variation in effluent N isotopic composition *among* treatment plants has received less attention (Anisfeld et al. 2007). Samples from the Jordan River were impacted by effluent from four POTW treatment plants, two of which (Central Valley Water Reclamation Facility and Salt Lake City Water Reclamation) were associated with substantially higher leaf $\delta^{15}N$ values than samples downstream of the two other treatment plants. These differences deserve further exploration, as they may be indicative of fundamental differences in treatment processes and N removal pathways during primary and secondary treatment.

The second pattern of more subtle (2–3‰) enrichment of leaf $\delta^{15}N$ values that we observed across many of the other urban reaches that were not influenced by POTW plants has multiple possible interpretations involving shifts in N sources and/or cycling. Plant assimilation of vehicular nitrogen dioxide (NO_2) in proximity to busy highways (e.g., tens of thousands of vehicles per day) can increase leaf $\delta^{15}N$ values (Ammann et al. 1999, Pearson et al. 2000, Redling et al. 2013). Here, we found no relationship between leaf $\delta^{15}N$ values and traffic density among several of the montane canyons adjacent to Salt Lake City, which varied in traffic from <5 to more than 5000 vehicles per day. Similarly, we found no spatial relationships between leaf $\delta^{15}N$ values and various indices of traffic and road density across the urban samples. These patterns do not exclude the possibility of dispersed but elevated urban NO_2 in explaining the widespread increase in urban leaf $\delta^{15}N$ values. Also, samples from Parley's Creek that were within 200 m of a major highway with approximately 50,000 vehicles per day had $\delta^{15}N$ values that were ~5‰ greater than nearby canyons, although we cannot rule out impacts from upslope suburban development with septic systems. Previous and ongoing work in the Salt Lake and Cache Valleys (Hall et al. 2014; Hall et al., *unpublished data*) showed similar bulk (wet + dry) N deposition fluxes and isotope composition across these montane to urban gradients. Thus, variation in N deposition fluxes or their isotopic composition is unlikely to explain increased urban leaf $\delta^{15}N$ values.

Inorganic fertilizer often represents one of the largest N sources to urban catchments (Groffman et al. 2004, Hale et al. 2014), and could contribute to the widespread urban increases in leaf $\delta^{15}N$ observed here. Salt Lake County non-farm fertilizer purchases represented a total input of approximately 7 kg N $ha^{-1}y^{-1}$ (Gronberg and Spahr 2012) relative to bulk (wet + dry) N deposition inputs of approximately 4 kg ha^{-1} y^{-1} (Hall et al., *unpublished data*). Although most inorganic fertilizer has $\delta^{15}N$ values between −2 and 2‰ (Bateman and Kelly 2007), it can represent a downstream source of isotopically enriched N as a consequence of fractionation during denitrification (Diebel and van der Zanden 2009, Atkinson et al. 2013) or ammonia volatilization. Denitrification often provides a significant N loss pathway in urban lawns and riparian zones (Groffman et al. 2002, Raciti et al. 2011). However, no consistent differences in leaf

$\delta^{15}N$ were observed in sites adjacent or downstream of golf courses or large public parks adjacent to the urban streams, where we would expect greater fertilizer inputs.

Diffuse inputs of $\delta^{15}N$-enriched human waste from leaking subterranean sewers, as observed in other cities (Kaushal et al. 2011, Divers et al. 2014), could also explain increased urban leaf $\delta^{15}N$ values. In particular, similarities in plant $\delta^{15}N$ values between Heber Valley urban ditches and the Jordan River (which received POTW effluent) highlight the possibility of leaky sewage infrastructure in the Heber Valley. Overall, the mechanism of widespread urban leaf $\delta^{15}N$ enrichment observed across all three valleys was potentially consistent with N derived from diffuse inputs of human/animal waste, increased inorganic fertilizer inputs that were fractionated prior to plant uptake in the riparian zone, or elevated urban NO_2.

Variation in leaf $\delta^{15}N$ values among agricultural reaches

Previous studies often showed similar increases in $\delta^{15}N$ values when comparing urban and agricultural ecosystems with natural areas (Anderson and Cabana 2006, Kohzu et al. 2008, Peipoch et al. 2012). Presumably, nutrient effluent from agricultural systems often represents an isotopically-similar mixture of animal waste and fractionated inorganic fertilizer as found in urban areas. Here, we found greater mean leaf $\delta^{15}N$ values in agricultural reaches than urban reaches, even when considering the very high values downstream from urban POTW plants. This could reflect the importance of animal waste inputs to the agricultural waterways, as well as the fact that major portions of the urban waterways had relatively low N availability and consequently less opportunity for fractionating N losses. For illustration, mean annual baseflow total N concentrations measured 0.31 ± 0.36 and 0.34 ± 0.14 mg l^{-1} along the urban reaches of Red Butte Creek and the Logan River, respectively, relative to 4–8 mg l^{-1} in the Jordan River, which receives treated POTW effluent (M. Baker, *unpublished data*). High variability (6‰) in leaf $\delta^{15}N$ values among agricultural stream reaches in the Cache Valley suggested the importance of management variation, likely related to animal waste inputs.

Variation in montane leaf $\delta^{15}N$: broader implications for environmental monitoring

A common theme that we observed in wildland montane canyons in both the Cache and Salt Lake Valleys was a relationship between the presence of people and animals—variously cattle, elk, or dogs—and increased leaf $\delta^{15}N$ values relative to reference catchments. Craine et al. (2009*a*) found a similar but smaller (1 ‰) enrichment in leaf $\delta^{15}N$ values with an increase in grazing intensity in a South African grassland. These patterns likely reflect volatilization of ^{15}N-depleted NH_3 from animal urine and manure, and consequent ^{15}N enrichment of the soil/plant system (Frank et al. 2004). They also imply that leaf $\delta^{15}N$ of riparian vegetation could provide a potential tool for addressing the N-cycling impact of stocking rates in wildland grazing areas and animal access in recreational areas. Similarly, leaf $\delta^{15}N$ values also reflected the impact of humans at the wildland/urban interface in the Salt Lake Valley canyons. The consistently large (~4‰) increase in leaf $\delta^{15}N$ values in a suburban montane reach of Emigration Creek lacking municipal sewers was strongly indicative of a diffuse septic waste N source that varied little along the reach, while a ~2‰ increase adjacent to a campground in Big Cottonwood Canyon was also likely due to human waste. For illustration, a two-source mixing model between montane N sources and human waste N (conservatively assuming waste values of 20‰) implied that at least 20% of plant N was derived from septic effluent in Emigration Canyon.

Finally, the impact of relatively modest human activities and land use alterations (e.g., pets, livestock, highways) on leaf $\delta^{15}N$ values in otherwise undeveloped wildland montane catchments is important to consider in light of previous work that used plant and soil $\delta^{15}N$ values as metrics of N cycling in "natural" ecosystems (Amundson et al. 2003, Craine et al. 2009*b*, Houlton and Bai 2009). These studies primarily assessed variation in leaf $\delta^{15}N$ values as a function of climate. For example, a global regression of leaf $\delta^{15}N$ values vs. mean annual temperature implies that a ~10°C increase in temperature would correspond with a 1.5‰ increase in leaf $\delta^{15}N$ values (Amundson et al. 2003). Here, leaf $\delta^{15}N$ values showed no rela-

tionship with elevation (a proxy for climate) across a 1000 m gradient after accounting for land use variation. Rather, we observed large differences in leaf $\delta^{15}N$ values across wildland montane watersheds in response to relatively subtle management differences, suggesting that these factors could contribute to the substantial residual variance in leaf $\delta^{15}N$ values often observed in large-scale studies.

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Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/ES15-00319.1.sm