



Elevated stream inorganic nitrogen impacts on a dominant riparian tree species: Results from an experimental riparian stream system

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[1] The release of inorganic nitrogen from intensive agricultural practices and urbanization has resulted in significant alterations of the aquatic nitrogen cycle in riparian ecosystems. Nevertheless, impacts of stream nitrogen inputs on the terrestrial nitrogen cycle and the water and carbon cycles are unclear. Information on terrestrial ecosystem responses to stream N loading is largely absent in part because of the difficulty in controlling for temporal and spatial variation in streamflow, geomorphology, climate, and vegetation. To address these issues, we constructed a dual-plot artificial stream riparian system within a 10-year-old plantation of a dominant riparian tree species, box elder (*Acer negundo*). The dual-plot design allowed for different concentrations of stream inorganic nitrogen between plots while controlling for ecohydrologic and geohydrologic variability. The system was used to investigate elevated inorganic stream nitrogen impacts on water use patterns, above-ground productivity, and leaf chemistry of streamside box elder trees over two consecutive growing seasons (2006 and 2007). One plot received inorganic soluble fertilizer that brought the NO_3 concentration of stream water from $5 \mu\text{mol l}^{-1}$ to about $100 \mu\text{mol l}^{-1}$, while the second plot received no additional nitrogen. Relative stem sap flux density (J_s) did not vary between plots until near the conclusion of the 2006 growing season, when trees in the fertilized plot showed a steep upswing in J_s relative to trees in the control plot. Sap flux in 2007 increased consistently by $0.4\% \text{ day}^{-1}$ in the fertilized plot relative to the control plot over a 75-day period, before leveling off near the conclusion of the growing season. At the onset of the experiment, leaf nitrogen per unit mass and leaf nitrogen per unit area were significantly higher in the control plot, and leaf C:N ratios were lower. In 2007, however, differences in leaf chemistry disappeared, suggesting that leaf nitrogen increased in the fertilized trees relative to the control trees. Stem diameter growth in 2007 was 15% greater in the fertilized trees, although there were no differences in either canopy radial or canopy height growth throughout the experiment. Results from this investigation suggest that increases in stream inorganic nitrogen affect water use, litter quality, and productivity of dominant riparian vegetation. These effects may have important feedbacks on several ecohydrological processes.

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1. Introduction

[2] Anthropogenic land use and land use change have had, and continue to have considerable impacts on stream nitrogen (N) concentrations on a global scale [Jordan and Weller, 1996; Howarth *et al.*, 2002]. The amount of N transported by the world's rivers into the oceans has roughly doubled and rates of N transported from developed areas have increased as much as 50 times as a result of human

activity since the industrial revolution [Meybeck, 1982]. In the United States, stream nitrogen inputs from human activity doubled from 1961 to 1997 [Howarth *et al.*, 2002]. Stream nitrogen concentrations are correlated with proportion of agriculture land in a catchment, because of non-point source fertilizer runoff [Howarth *et al.*, 2002]. Likewise, stream N concentrations are generally greater in river systems draining more densely urbanized catchments [Jordan and Weller, 1996].

[3] Land use induced changes in stream N concentrations may have a considerable effect on riparian vegetation and ecosystem processes. Nitrogen is a key element limiting NPP and NEP in many terrestrial ecosystems [Schuur and Matson, 2001; Mack *et al.*, 2004; Belnap *et al.*, 2005], including riparian systems where water is often not limiting. Dissolved inorganic N (DIN, i.e., NO_3 and/or NH_4) in

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groundwater and stream water can become a significant source for N uptake by streamside vegetation [Schade *et al.*, 2002]. The amount of plant uptake of N is likely related to concentrations of N in surface and groundwater, N mineralization rates in riparian soils [Schade *et al.*, 2002], and root activity in shallow soils versus groundwater and/or the capillary fringe. Riparian habitats that are dominated by phreatophytic tree species such as cottonwood (*Populus* spp.), willow (*Salix* spp.), and box elder (*Acer negundo*), typically have the highest potential to remove water and DIN from stream and groundwater systems because their relatively deep roots often tap the shallow groundwater table. In agricultural watersheds, for example, riparian buffer strips of cottonwood and willow have successfully been added to remove large amounts of N in runoff from fertilized fields, thereby improving stream water quality [Lowrance, 1998; Yamada *et al.*, 2007].

[4] While there is considerable evidence that riparian vegetation acquires N from stream water and groundwater, it is unclear whether higher stream N concentrations result in greater rates of transpiration and productivity of riparian vegetation. Likewise, feedbacks between elevated stream N concentrations and ecohydrological processes such as evapotranspiration (ET), subsequent streamflow, carbon cycling, and other nutrient cycles are also unclear. Nitrogen fertilization typically increases leaf area, growth, and water use of tree stands that are not water limited [Ewers *et al.*, 1999, 2001; Phillips *et al.*, 2001; Hubbard *et al.*, 2004]. In general, higher leaf area is associated with higher water use rates [Sala *et al.*, 1996; Hubbard *et al.*, 2004], although increases after canopy closure may be small because of reduced canopy light interception and increased boundary layer resistance [Jarvis and McNaughton, 1986; Martin, 1989]. Fertilization may also impact relative allocation to leaves and absorptive roots, resulting in higher leaf area to absorptive root area ($A_l:A_r$) ratios [Gower *et al.*, 1992; Giardina *et al.*, 2003], and lower water uptake capacity relative to transpiration capacity [Sperry *et al.*, 1998; Hacke *et al.*, 2000]. Therefore increased stream DIN concentrations could result in lower ET and productivity of riparian trees if increases are met with reduced water availability.

[5] The relationship between stream N and the structure and function of riparian vegetation have added importance in riparian systems that receive heavy inputs of N from agricultural runoff and urbanization. Nevertheless, information on the ecohydrological consequences of stream N loading is largely absent, in part because of the difficulty in controlling for confounding variables in time and space. For example, field studies assessing the effect of stream N on riparian ecosystem function have relied on comparisons of separate streams or reaches that not only vary in N content, but also other physical, chemical or biological parameters [Ferreira *et al.*, 2006].

[6] In this paper we describe the construction and utilization of an artificial, dual-plot stream system built specifically to address impacts of stream DIN concentrations on riparian ecosystem function and productivity in the Intermountain West. The system is unique in that it allows for multiple long-term investigations, while controlling for changes in genetic composition of trees, streamflow, geomorphology, and riparian habitat. We present the results of

a two-year investigation of the relationship between enhanced stream DIN concentrations and growth, leaf chemistry and transpiration fluxes of replicated clones of mature streamside box elder trees (*A. negundo* Sarg.). Soluble ammonium nitrate was injected into one of the stream systems to raise the NO_3^- concentration from approximately $5 \mu\text{mol l}^{-1}$ to roughly $100 \mu\text{mol l}^{-1}$, a typical concentration for stream reaches along the Wasatch Front in northern Utah that have received heavy inputs of DIN from agricultural or urban activities. We hypothesized that mature streamside box elder trees that received enhanced stream DIN would result in (1) increased stem sap flux rates, (2) increased above-ground net primary productivity, and (3) markedly increased leaf N and decreased leaf C:N ratios compared to streamside box elder trees that received no stream DIN addition.

2. Materials and Methods

2.1. Site Description

[7] The artificial stream system was constructed at the University of Utah, Department of Biology Growth Site at the east end of campus ($40^\circ 45' 39.25''\text{N}$, $111^\circ 49' 48.80''\text{W}$, 1481 m). The growth site consists of a 100 m by 40 m open field of loamy soil. The upper 2 m of topsoil had been transported to the site a decade earlier, and therefore contained no soil horizons. Box elder cuttings collected from the nearby Red Butte Canyon Research Natural Area were potted in a glasshouse in 1997 and allowed to take root. The cuttings were taken from a single location in order to minimize genotypic variation. A total of 36 cuttings were transplanted at the growth site the following year three meters apart in a six by six grid. A total of 33 cuttings successfully established and have now reached reproductive maturity (the remaining three trees died shortly after transplanting).

[8] Volumetric soil moisture (θ) was monitored with Campbell CS 616 water content reflectometer (WCR) probes (Campbell Scientific, Logan, UT). The WCR probes were installed in two vertical profiles in open trenches at 15-, 30-, 60-, and 90-cm depths were then immediately backfilled (Figure 1). Sensor output was logged every 30 s and stored as 30 min averages with a Campbell CR10X-2M data logger (Campbell Scientific, Logan, UT, USA). The output period was converted to volumetric soil water content using a factory derived linear calibration equation.

[9] Relative humidity, air temperature, photosynthetic active radiation, and precipitation were measured continuously from a micrometeorological station located 50 m from the artificial stream system. Relative humidity and air temperature were measured with a Vaisala HMP 45 AC humidity and temperature probe (Vaisala, Woburn, MA, USA), placed approximately 2 m above the ground surface. Photosynthetic active radiation (Q) was measured with a Li-Cor LI-190SZ quantum sensor (Li-Cor, Lincoln, NE, USA) placed approximately 3 m above the ground surface. Precipitation was measured with a Texas Electronics TE525 tipping bucket rain gauge (Texas Electronics, Inc., Dallas, TX). Micrometeorological data were measured every 30 s and stored as 10 min averages with a Campbell CR10X-2M data logger (Campbell Scientific, Logan, UT, USA). Measurements of relative humidity and air temperature

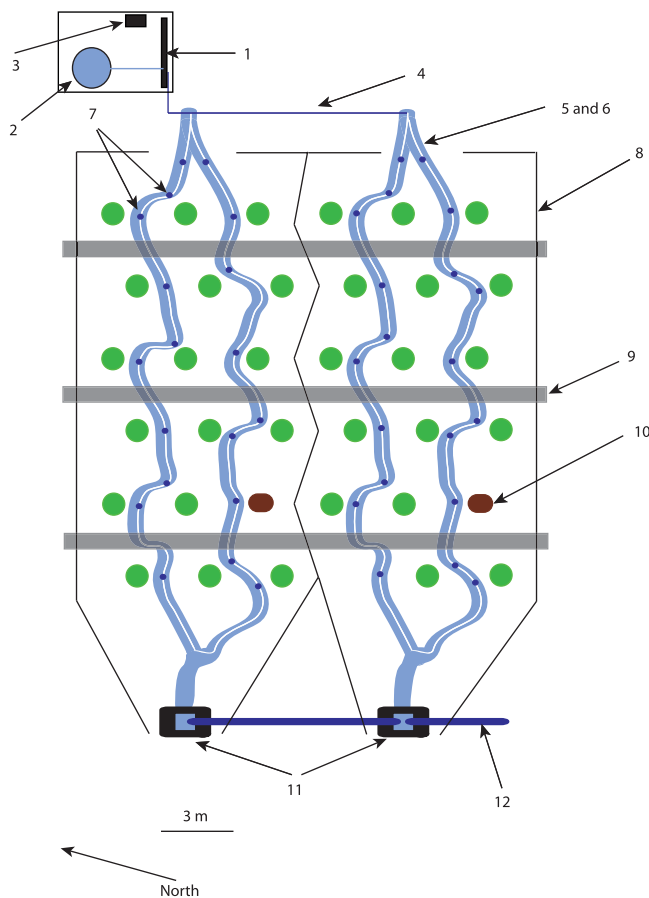


Figure 1. Schematic of the experimental riparian stream system constructed in 2005 at the University of Utah campus in Salt Lake City, UT. A detailed description of each numbered component (shown with arrows) are as follows: (1) Plumbing manifold: Valve system to control stream flow and injection rate from nitrogen tank. (2) Nitrogen tank: 175 g (662 l) tank that stores NH_4NO_3 solution. (3) Data logger enclosure: Enclosure that houses a Campbell CR 23X data logger for recording stream flow and nitrogen injection rates. (4) PVC piping: 0.75" (1.905 cm) dia. PVC piping that brings groundwater from plumbing manifold to artificial stream channels. (5) Stream channels: 1 m wide \times 0.25 deep stream channels constructed to transport groundwater through plot. (6) PVC piping in stream: 0.75" (1.905 cm) dia. PVC piping that runs in a closed loop throughout the stream bed. (7) Adjustable bubblers: 360 degree adjustable bubblers that directly feed water from PVC into the stream channels. (8) PCV plot dividers: 0.25" (0.635 cm) thick PVC sheets inserted vertically into the soil profile. The sheets were fabricated into single units using solvent acrylic glue and wood screws. (9) Elevated walkways: 0.6 m wide walkways constructed of treated wood and grated steel mounted on cement blocks. (10) Soil moisture monitoring: Campbell CS 616 WCR probes inserted into two vertical profiles at 15, 30, 60, and 90 cm soil depths. (11) Stream catch basins: 50 l drainage basins constructed of plastic tubs placed 50 cm below the depth of the streams. (12) Outflow piping: 6" (15 cm) dia. flexible corrugated tubing that drained the catch basins into a lowland area away from the stream system.

were used to calculate atmospheric vapor pressure deficit (vpd).

2.2. Construction of the Artificial Stream System

[10] The artificial stream system was constructed in the spring of 2005 in four primary phases: (1) burying PVC sheets to isolate plots, (2) construction of the streams, (3) construction of the plumbing system to feed the streams with water and nitrogen, and (4) construction of walkways to minimize soil disturbance (see Figure 1 for schematic of stream system).

[11] Two plots were established within the stream system to allow for a control plot (no stream nitrogen addition), and an experimental plot with nitrogen addition. Plots were established by digging 0.5 m wide, 2 m deep trenches with a small backhoe. It was determined from the trenching that rooting depth of all the box elder trees was within the upper one meter of the soil profile (depth to the water table is greater than 20 m). Thus trenching was well below rooting depth. Trenching occurred down the center of the box elder plantation, resulting in two plots: one north of the trench having 17 trees, and the other south of the center trench having 16 trees. Trenching also occurred down the outside of both plots, and at both ends of each plot, while leaving openings at both ends to plumb water and nitrogen inputs and outputs (Figure 1). Two WCR probes (see above) were installed at the bottom of the center trench at the east and west ends to determine whether water from the running streams infiltrated below the depth of the PVC sheets.

[12] Immediately after trenching, 0.25" (0.635 cm) thick PVC sheets (SABIC Polymershapes, Salt Lake City, UT) were installed into the open trenches. The PVC sheets were originally purchased in 4' \times 8' (1.2 \times 2.4 m) sheets. The sheets were cut to length so that they extended approximately 15 cm above the ground surface to prevent surface runoff between plots. The sheets were then fastened together in sections (three sheets per section) using solvent-based acrylic glue and installed into the trenches. The sections were then fastened with sheet metal screws. In some cases the sheets were bent at 45 or 90° angles, such as at both ends of the plots (see Figure 1). In these cases, they were heated with a heat gun, and carefully bent to the correct angle before installing into the trenches. After the PVC sheets were installed, they were allowed to settle for approximately 24 hours before backfilling the trenches with hand shovels.

[13] Streams were constructed to bring free-flowing water, pumped from a municipal water source, to within one meter of each tree (Figures 1 and 2). Two streams of approximately 1 m width and approximately 0.2 to 0.25 m depth were constructed in each plot with a small backhoe. Because there was already a gentle slope from the top (inflow) to the bottom (outflow) of both plots, it was not necessary to construct progressively deeper streams from top to bottom. The two streams in each plot were joined at the top (inflow) and bottom (outflow), effectively resulting in a single stream per plot (Figure 1). The streams were lined with a thin layer of gravel to reduce sediment transport. Water was fed by running 0.75" (1.905 cm) diameter PVC pipe along the center for the entire length of the streams (Figures 1 and 2). The PVC configuration incorporated a T-junction at the inflow where the two



Figure 2. Photo looking east of the experimental riparian system constructed in 2005 on the campus of the University of Utah in Salt Lake City, UT (see Figure 1 for detailed description).

streams in each plot separated, and were rejoined where the two streams converged near the outflow so that each plot was fed water from a single irrigation unit (Figure 1). Water was delivered from the PVC piping to the stream from 360° adjustable bubblers. A single bubbler was placed at the top of each stream system, and near every tree in each plot (Figure 1). This allowed the system to be adjusted so that all trees could receive the same volume of water. However, in the present study, water was only delivered from the uppermost bubbler, so that diurnal streamflow variation could be monitored. The outflow consisted of a 50 l catch basin at the bottom of each plot. Fifteen cm diameter flexible corrugated tubing drained the catch basins into a large lowland area near Red Butte Creek.

[14] Irrigation supply and nitrogen injection into the stream system were controlled and monitored through an intricate plumbing manifold. Manual control valves at the manifold allowed for simultaneous adjustment of the water supply to each plot from 0 to 32 l min⁻¹. Although, the system could easily be operated to adjust streamflow to mimic phases of snowmelt/runoff, stormflow, and base flow, we operated the system at a constant flow rate of 16–18 l min⁻¹ so that we could focus the experiment on stream nitrogen impacts on ecosystem function. The streams were operated in 2006 and 2007 from late April, just before leaf flush until early November, well after leaf senescence commenced. Volumetric streamflow in each plot was monitored with a Dwyer, SFI-801-3/4-A711 flow meter/transmitter (Dwyer Instruments, Michigan City, IN). Each flow meter was individually calibrated against a Sensus SR 2 magnetic drive water meter (Sensus Metering Systems, Raleigh, NC) at flow rates between 10 and 60 l min⁻¹. After calibration, the flow meters were installed into the plumbing manifold and connected to a Campbell CR23X datalogger (Campbell Scientific, Logan, UT). Data were logged every 30 s and stored as 30 min averages.

[15] Three elevated walkways were constructed across the plots to minimize soil disturbance during research activities (Figure 2). The walkways were constructed from treated 4" × 4" (10.15 × 10.15 cm) lumber mounted on cement blocks, creating a 0.6 m wide frame that spanned the length of the two plots. Grated steel was then stapled to the wood frame providing a sturdy walkway across the plots. The

grated steel allowed for enough sunlight and air to pass under the walkways to prevent significant microenvironmental gradients.

2.3. Stream Nitrogen Treatment

[16] Nitrogen was injected into the irrigation manifold using a Dosatron DI16 solution injection system (Dosatron, Clearwater, FL). The DI16 allowed for adjustable injection rates from 1:500 (0.2%) to 1:64 (1.6%) of streamflow. The injector was installed directly into the plumbing manifold, which allowed nitrogen solution to be introduced to either stream system through a network of locking valves. The north plot was randomly selected to receive nitrogen from the injector, while the south plot served as a control plot. Quarter inch (0.635 cm) inside diameter Tygon tubing was connected to the DI16 injector with the other end inserted into a 175-gallon (662 l) plastic tank. The tank was filled with water and 34-0-0 water-soluble, 17% NH₄, 17% NO₃ fertilizer was added. A small electric pump was placed in the tank to keep the nitrogen well mixed. The adjustable injection rate allowed the nitrogen concentration in stream water to be adjusted by either varying the injection rate or the concentration of nitrogen in the tank. The injection rate was monitored with a Dwyer TF1032 turbine flow sensor (Dwyer Instruments, Michigan City, IN). The flow sensor was calibrated by running water through the sensor's turbine, while collecting the outflow in a graduated cylinder over a measured rate of time. Flow rates were calibrated incrementally from 3 l hr⁻¹ to 12 l hr⁻¹. The flow sensor was installed to the Tygon tubing between the Dosatron and the nitrogen water tank, and connected to a Campbell 23X datalogger (Campbell Scientific, Logan, UT). Injection rates were logged every 30 s and stored as 30 min averages.

[17] The injection rate and nitrogen concentration in the tank were adjusted to bring the stream nitrate concentration from a background of about 5 μmol l⁻¹ to 100 μmol l⁻¹, a concentration that is typical for urban stream systems in northern Utah. Nitrogen was added to the north plot from early May until early October in 2006 and 2007. Stream NO₃ concentrations were periodically measured using methods outlined by *Nydahl* [1976] and *Parsons et al.* [1984]. In 2007, a ¹⁵N tracer was added to the fertilizer to bring the δ¹⁵N of NH₄ and NO₃ in stream water from a measured value of -0.2‰ (in reference to a known atmospheric standard) to approximately +200‰. The label allowed us to determine whether the box elder trees were acquiring stream nitrogen. To achieve a 200‰ label, one gram of 10 atom% ¹⁵NH₄/¹⁵NO₃ (ICON Isotopes, Summit, NJ) was mixed with every 800 g of fertilizer added to the irrigation system. The ¹⁵N label was added to the fertilizer from early May through early August.

2.4. Stem Sap Flux Measurements

[18] Granier-type temperature sensors [*Granier*, 1987; *Hultine et al.*, 2007] were constructed in the laboratory to measure stem sap flux density (J_s , g H₂O m⁻² sapwood s⁻¹) of the box elder trees. Each sensor consisted of a pair of 10 mm long, 20 mm diameter stainless steel probes inserted approximately 15 cm apart along the axis of the hydroactive xylem (i.e., sapwood). The azimuth direction of each sensor was randomly selected to eliminate potential biases due to non-uniformity in sap flux around the stem. The upper probe

(i.e., toward the canopy) was supplied with constant heat of 200 mW, and the temperature difference between the heated probe and the lower, unheated reference probe was converted to sap flux density according to *Granier* [1987]:

$$J_s = 0.0119 \left(\frac{\Delta T_0}{\Delta T} - 1 \right)^{1.23} \quad (1)$$

where ΔT is the temperature difference between the heated and unheated probes and ΔT_0 is the temperature difference obtained under zero flow conditions. We assumed that zero flow only occurred at night when vapor pressure deficit was at or near zero.

[19] A single sensor was inserted into the main stem of each tree at approximately 1.0 m above the ground. Temperature differences of all sensors were logged every 30 s and stored as 30 min averages with a Campbell CR10X-2M data logger (Campbell Scientific, Logan, UT, USA). Mean daytime J_s was calculated in order to correlate with mean daytime vpd. Daytime was defined by the period when Q was greater or equal to $10 \mu\text{mol m}^{-2} \text{s}^{-1}$.

[20] Total daily and annual tree transpiration in each plot was calculated by multiplying J_s by sapwood area (A_s). However, because sap flux studies on these trees are ongoing, we have not yet established sapwood, area/stem diameter relationships due to the destructive nature of estimating sapwood depth. Therefore, sapwood area of each tree was estimated from allometric relationships between stem diameter and A_s determined on streamside box elder trees in Red Butte Canyon [Hultine *et al.*, 2007]. Stem diameter was determined as the midpoint between measurements taken at the conclusion of the previous growing season and measurements taken at the conclusion of the current growing season.

2.5. Leaf Mass per Area, Leaf $\delta^{13}\text{C}$, Leaf Nitrogen, and Relative Growth Rate Measurements

[21] Leaf carbon isotope ratios were measured ($\delta^{13}\text{C}$, ‰) to assess the relative balance between the supply of CO_2 via leaf conductance and the demand for CO_2 by photosynthetic enzymes. Specifically, decreases in stomatal conductance and/or increases in photosynthetic capacity (from increased leaf N content) will result in reduced fractionation against ^{13}C and less negative values of leaf $\delta^{13}\text{C}$ [Ehleringer, 1991]. Carbon isotope ratios of bulk leaf tissue, leaf nitrogen content per unit leaf mass (N_{mass} , mg g^{-1}), and nitrogen per unit leaf area (N_{area} , mg cm^{-2}) and leaf mass per area (LMA, g m^{-2}) were measured in leaves collected from each box elder tree in May, early July, and late August of 2006 and 2007. Fresh leaves were scanned with a high-resolution computer scanner, and one-sided leaf area was measured using public domain NIH image program software (<http://rsb.info.nih.gov/nih-image/>). Leaves were then oven-dried for 72 hours at 70°C and weighed to the nearest 0.01 g to obtain LMA. Leaves were ground to a fine powder and analyzed for nitrogen content and carbon isotope ratios using an elemental analyzer (Carlo Erba, Model 1108, Milano, Italy) coupled with a Finnigan MAT delta S isotope ratio mass spectrometer (San Jose, CA, USA) at the Stable Isotope Ratio Facility for Environmental Research (SIRFER)

Lab, at the University of Utah. Carbon isotope ratios were calculated using δ notation:

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (2)$$

where R is the ratio of the heavy isotope (^{13}C) to the lighter isotope (^{12}C), and the standard was V-PDB. The instrument error (twice the standard deviation) associated with each observation was $\pm 0.1\%$. Leaf nitrogen content per unit leaf area was calculated by multiplying N_{mass} by LMA.

[22] Relative growth rates of each tree were estimated by measuring the annual percent change in stem diameter, canopy height, and canopy radius (i.e., drip line) of each tree in the fall after leaf drop. Leaf area was determined allometrically by correlating stem diameter with total branch diameter of all branches extending from the main stem, then correlating branch diameter with total number of nodes, and finally calculating mean leaf area per node. Leaf area per node was determined by collecting leaves from eight nodes per tree, leaves were immediately dried and weighed using methods described above. The mean mass per node was then determined and the previously established LMA was used to calculate mean leaf area per node. Leaf area index (LAI, $\text{m}^2:\text{m}^{-2}$) was calculated by dividing total leaf area with canopy area determined from canopy radius measurements.

2.6. Background Soil Nitrogen, $\delta^{15}\text{N}$, and Carbon Content

[23] Background soil nitrogen content, $\delta^{15}\text{N}$, organic carbon content, and C:N ratios were determined from soil cores collected at 15–20 cm depths. Six cores (three per plot) were collected in the intercanopy spaces at a minimum distance of 2 m from the nearest stream. Soil samples were oven dried at 70°C for 72 hours and ground into fine powder. In order to remove inorganic carbon bound in soil carbonates, three grams of soil were separated from each sample and washed for over 48 hours with 50 ml of 0.5 M HCL. Soil organic carbon content and soil nitrogen were analyzed on washed and unwashed soils, respectively, using an elemental analyzer (Carlo Erba, Model 1108, Milano, Italy) coupled with a Finnigan MAT delta S isotope ratio mass spectrometer (San Jose, CA, USA) at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) Lab at the University of Utah.

2.7. Statistical Analysis

[24] Because of the constraints for building multiple plots (as often is the case in experimental ecosystem studies), we were only able to compare within-plot variation and not between-plot differences. In other words, the experimental design was limited to pseudo-replicated trees.

[25] Regression analysis was performed to detect relative changes in J_s between fertilized and control trees through time using analysis of variance (ANOVA). Regression analysis was performed to detect plot-scale differences in the response of J_s to vpd using analysis of covariance (ANCOVA). Mean plot differences in J_s , leaf traits and relative growth rates during individual sampling periods and

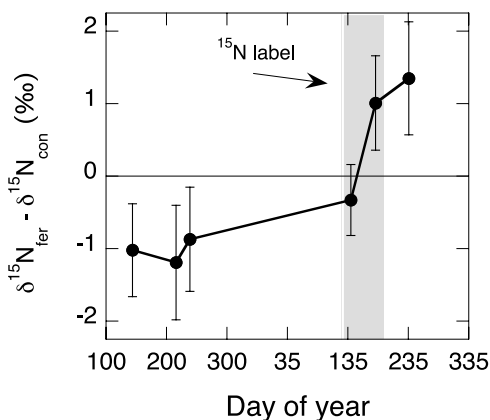


Figure 3. Leaf $\delta^{15}\text{N}$ content of box elder trees receiving stream NH_4NO_3 addition with a $\delta^{15}\text{N}$ isotopic label relative to trees receiving no nitrate addition. The $\delta^{15}\text{N}$ label was added to the NH_4NO_3 stream fertilizer from 11 May 2007 (DOY 132) through 14 July 2007 (DOY 195). The label brought the $\delta^{15}\text{NO}_3$ of the fertilized stream from approximately -0.2‰ to approximately 200‰ , while $\delta^{15}\text{NO}_3$ of the control stream remained about 2.0‰ . Error bars represent ± 1 standard error of the mean.

months were analyzed using a Student's t test. Mean seasonal differences between plots in mean daily sap flux scaled transpiration were analyzed using a Student's t test. Plot differences in the seasonal course of leaf traits were assessed by repeated measures analysis of variance (MANOVA) where multiple measurements on a given tree constituted the repeated variables [Potvin *et al.*, 1990]. In each analysis, the main effect of plot was tested as a between subjects effect. JMP 5.1 (SAS institute Inc, Cary, NC, USA.) was used for all statistical analysis, with $P \leq 0.05$ as the significance level.

3. Results

3.1. Leaf $\delta^{15}\text{N}$ Content and Background Soil N and C:N

[26] Mean bulk soil nitrogen content in the intercanopy spaces was 0.09% , with a standard error (SE) of 0.01 , while

mean C:N was 31.5 ($\text{SE} = 1.4$). Mean $\delta^{15}\text{N}$ of bulk soil was slightly, but not significantly, lower in the fertilized plot compared to the control plot: 6.2‰ ($\text{SE} = 0.6$) and 6.9‰ ($\text{SE} = 0.3$), respectively ($P = 0.37$, $n = 3$ soil samples per plot). At the onset of the experiment, mean leaf $\delta^{15}\text{N}$ of the fertilized plot was also lower (7.1‰ , $\text{SE} = 0.34$) compared to the control plot (8.2‰ , $\text{SE} = 0.30$) ($P = 0.0365$). The lower leaf $\delta^{15}\text{N}$ in the fertilized plot was maintained throughout the 2006 growing season (Figure 3). In 2007, a nitrogen isotope label was added to the NH_4NO_3 fertilizer to bring stream nitrate $\delta^{15}\text{N}$ from -0.2‰ to approximately 200‰ . Bulk leaf nitrogen, measured from leaves collected 10 days after the label was released, showed a slight relative shift in $\delta^{15}\text{N}$ between plots compared to 2006 (Figure 3). Leaves collected in July and August of 2007 showed a larger shift in leaf $\delta^{15}\text{N}$ compared to similar periods in 2006 (Figure 3), suggesting that the trees in the fertilized plots were acquiring stream nitrogen throughout the year. In July of 2007, leaf $\delta^{15}\text{N}$ was marginally higher in the fertilized trees than in the control trees ($P = 0.0585$), and was significantly higher in the fertilized trees in August ($P = 0.0366$).

3.2. Stem Sap Flux

[27] At the onset of the experiment, mean daily J_s was generally 10 to 20% lower in the fertilized plot ($J_{s,\text{Fert}}$) compared to the control plot ($J_{s,\text{Con}}$) (Figure 4a), and was significantly lower in May–June and July (Table 1). Sap flux density remained consistently lower in the fertilized plot until early August, around day of year 220. By late August, J_s ranged from about 10 to 25% higher in the fertilized plot compared to the control plot (Figure 4a). There was a significant, linear relationship in 2006 between relative J_s ($J_{s,\text{Fert}}:J_{s,\text{Con}}$) and time, although the fraction of the variance explained was modest ($R^2 = 0.21$, $F = 27.7$, $P < 0.0001$, $n = 107$ days). However, a second-order polynomial relationship explained more of the variation than the linear relationship ($R^2 = 0.36$, $F = 28.9$, $P < 0.0001$), incorporating the late season upswing in $J_{s,\text{Fert}}:J_{s,\text{Con}}$ (Figure 4a).

[28] In early 2007, J_s was again lower in the fertilized trees, but by only 5 to 10% compared to the trees in the control plot (Figure 4b), and these differences were not significant in May–June (Table 1). By 14 June (DOY 165),

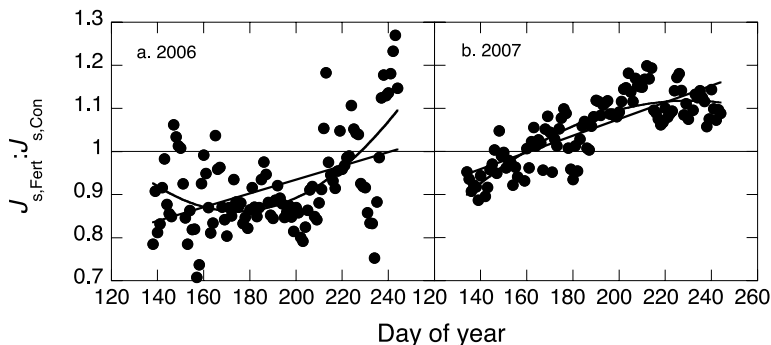


Figure 4. Relative difference in mean daytime stem sap flux density (J_s , $\text{g m}^{-2} \text{s}^{-1}$) in box elder trees receiving stream NH_4NO_3 addition relative to trees receiving no NH_4NO_3 addition during the 2006 and 2007 growing seasons. (a) Relationship between mean J_s of fertilized trees relative to control trees from 18 May (DOY 138) to 1 September (DOY 244) 2006. (b) Relationship between mean J_s of fertilized trees relative to control trees from 14 May (DOY 134) to 1 September (DOY 244) 2007. Both panels show linear and second-order polynomial regressions.

Table 1. Mean Daytime Sap Flux ($\text{g m}^{-2} \text{s}^{-1}$) of Box Elder Trees and Mean Daytime Atmospheric Vapor Pressure Deficit (kPa) at an Experimental Riparian System in Salt Lake City, UT During the 2006 and 2007 Growing Seasons^a

Month	2006				2007			
	Fertilized	Control	<i>P</i>	vpd	Fertilized	Control	<i>P</i>	vpd
May–June	44.7 (1.38)	50.9 (1.53)	0.0035	2.21	58.7 (1.98)	59.7 (2.03)	0.73	2.47
July	38.0 (0.64)	43.3 (0.70)	<0.0001	3.07	51.8 (1.01)	47.0 (1.00)	0.0015	3.34
August	39.5 (1.13)	39.4 (0.96)	0.92	2.63	45.6 (0.65)	41.4 (0.66)	<0.0001	2.92

Bold data indicate significant differences at $P \leq 0.05$.

^aFertilized trees received stream NH_4 and NO_3 addition, while control trees received stream water without nitrogen addition. Probability values (*P*) of plot differences in are from J_s pooled mean values during each period.

J_s was consistently higher in the fertilized trees, and remained higher throughout the growing season. There was a significant linear relationship between $J_{s,\text{Fert}}:J_{s,\text{Con}}$ and day of year, which explained a large fraction of the observed variance ($R^2 = 0.63$, $F = 178.0$, $P < 0.0001$). A second-order polynomial regression again explained more of the variance than a linear regression ($R^2 = 0.71$, $F = 126.0$, $P < 0.0001$). In other words, $J_{s,\text{Fert}}:J_{s,\text{Con}}$ increased throughout the growing season until the end of July (about day of year 210) where differences in J_s between plots leveled off (Figure 4b). Mean differences in J_s were significant in both July and August (Table 1).

[29] Sap flux was generally higher in both plots in 2007 relative to 2006 (Table 1). Sap flux density increased from 2006 to 2007 in the fertilized plot 31, 37, and 15% in May–June, July, and August, respectively, while in the control plot, J_s increased 17, 5, and 5% in May–June, July, and August respectively. The higher sap flux in 2007 may be a consequence of higher mean monthly daytime vpd that occurred in 2007 relative to 2006 (Table 1). In habitats where soil water is non-limiting (i.e., along streamside), patterns of plant water use result largely from atmospheric drivers such as vapor pressure deficit and sunlight. However, in 2006 J_s was not well correlated with vpd in either plot (data not shown), suggesting that stomatal conductance may have been primarily limited by soil water deficits and not atmospheric water deficits. Conversely, mean daytime J_s in 2007 from trees in both plots was well correlated with daytime vpd (Figure 5), suggesting that the tree roots were tightly coupled to soil water. The relationship in 2007 between sap flux density (J_s) and vpd was non-linear, and was therefore fitted with an exponential saturation model:

$$J_s = b_1(1 - e^{-b_2 \text{vpd}}) \quad (3)$$

where b_1 and b_2 are fitting parameters. Early in the growing season (May–June), the model explained 86 and 81% of the variability in J_s of trees in fertilized and control plots, respectively ($P < 0.0001$ for fertilized and control trees) (Figure 5a). In July, the model explained 54 and 66% of the variability of J_s in fertilized and control plots, respectively ($P < 0.0001$ for fertilized and control trees) (Figure 5b), and in August, the model explained 52 and 67% of the variability of J_s in fertilized and control plots, respectively ($P < 0.0001$ for fertilized and control trees) (Figure 5c).

[30] Plot-scale differences in the response of J_s in 2007 to vpd were tested using analysis of covariance. In May–June, J_s was highly correlated with vpd ($F = 271.0$, $P < 0.0001$), and remained highly correlated in July ($F = 101.4$, $P < 0.0001$), and August ($F = 75.6$, $P < 0.0001$). In May–June,

there were no differences in the response of J_s to vpd between trees in the fertilized and control plots ($P = 0.49$, Figure 5a). Conversely, there were sharp differences be-

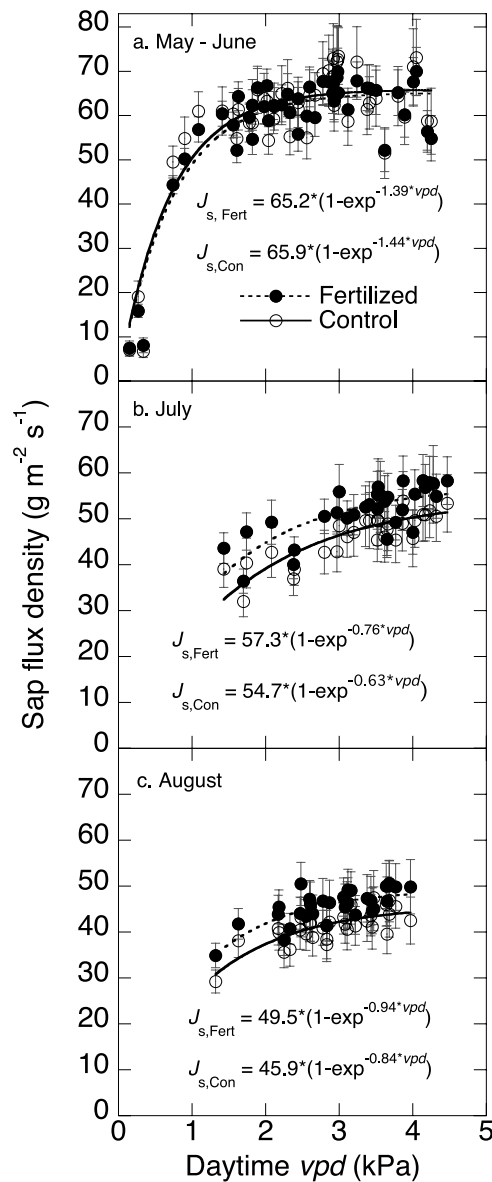


Figure 5. Relationship between mean daytime stem sap flux density (J_s) and mean daytime vapor pressure deficit (vpd) in box elder trees receiving stream NH_4NO_3 addition and trees receiving no NH_4NO_3 addition during the 2007 growing season. Data were collected from 14 (a) May to 30 June, (b) 1 to 31 July, and (c) 1 to 31 August.

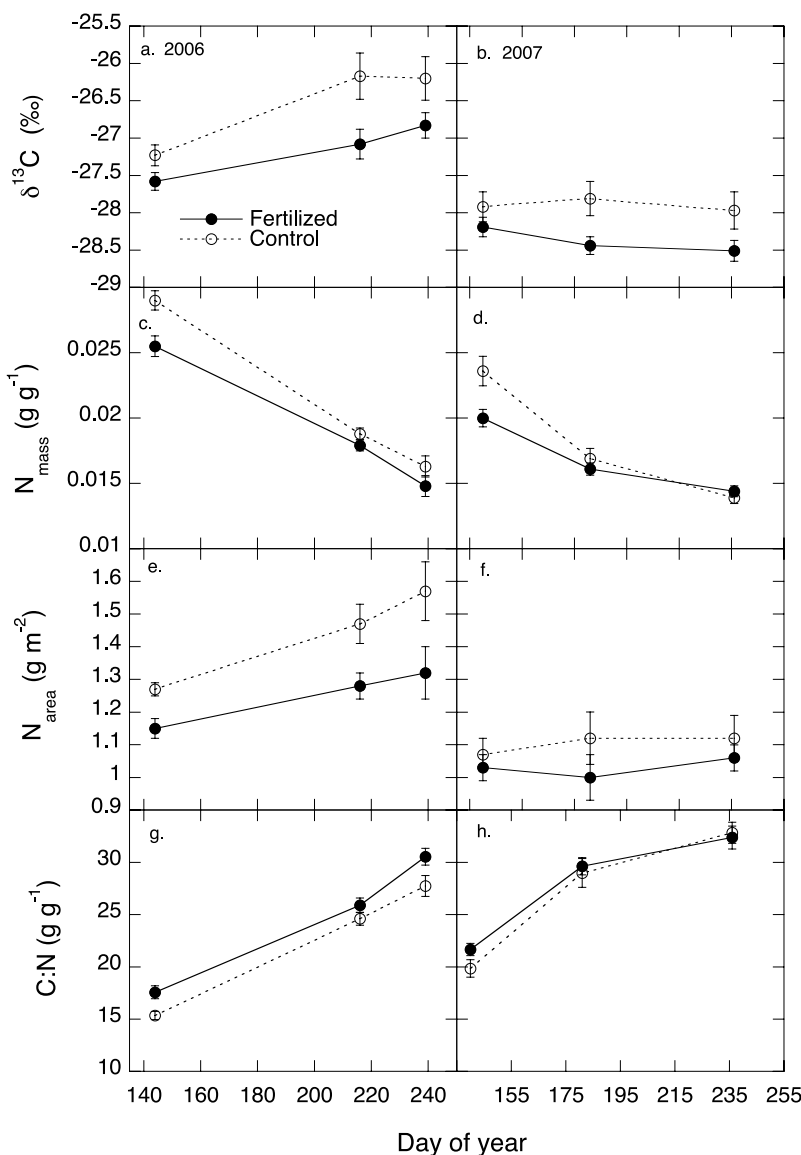


Figure 6. Patterns of leaf carbon isotope ratios ($\delta^{13}\text{C}$), bulk leaf nitrogen per unit leaf mass (N_{mass}), bulk leaf nitrogen per unit leaf area (N_{area}), and bulk carbon to nitrogen ratios (C:N) of fertilized (solid circles) and control (open circles) box elder trees during the 2006 and 2007 growing seasons at the experimental riparian system near Salt Lake City, UT. Error bars represent ± 1 standard error of the mean.

tween plots in the response of mean J_s to vpd in July ($F = 29.6$, $P < 0.0001$, Figure 5b) and August ($F = 45.8$, $P < 0.0001$, Figure 5c). There were no significant interactive effects (plot*vpd) throughout the 2007 growing season, meaning that despite differences in the absolute response to vpd, the slopes were similar.

3.3. Leaf $\delta^{13}\text{C}$, Leaf Nitrogen, Leaf Area, Relative Growth Rates, and Sap Flux Scaled Transpiration

[31] Bulk leaf $\delta^{13}\text{C}$ was slightly lower (more negative) during both the 2006 and 2007 growing seasons in the fertilized plot compared to the control plot ($P = 0.0085$ in 2006, and $P = 0.04$ in 2007, Table 2; Figures 6a and 6b). Mean monthly leaf $\delta^{13}\text{C}$ in the fertilized plot was -27.2 (0.12) and -28.4 (0.10) ‰ in 2006 and 2007, respectively. In the control plot, $\delta^{13}\text{C}$ was -26.5 (SE = 0.14) and -27.9 (SE = 0.11) ‰ in 2006 and 2007, respectively. In 2006, leaf

$\delta^{13}\text{C}$ increased (i.e., became more positive) through the growing season ($P < 0.0001$, Table 2; Figure 6a), while there was no change through the 2007 growing season ($P =$

Table 2. Probability Values From Repeated Measures (MANOVA) of $\delta^{13}\text{C}$, N_{mass} , N_{area} , and C:N Ratios of Box Elder Leaves Collected at an Experimental Riparian System in Salt Lake City, UT^a

	2006	$\delta^{13}\text{C}$	N_{mass}	N_{area}	C:N
Plot	0.0085		0.0047	0.0022	0.0061
Month	<0.0001		<0.0001	<0.0001	<0.0001
Plot*Month	0.24		0.09	0.44	0.49
2007					
Plot	0.04		0.29	0.44	0.55
Month	0.21		<0.0001	0.46	<0.0001
Plot*Month	0.11		0.16	0.96	0.33

Bold data indicate significant differences at $P \leq 0.05$.

^aLeaves were collected in May, July, and August of 2006 and 2007.

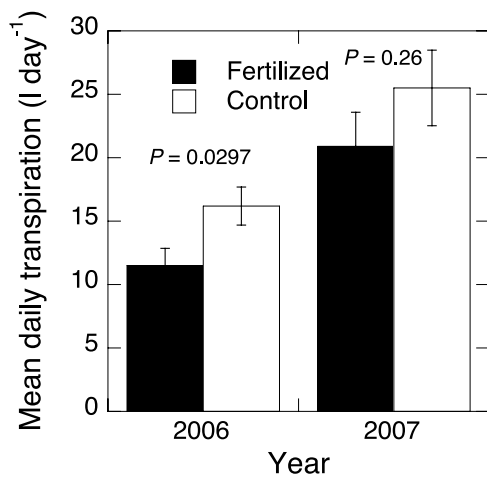


Figure 7. Mean daily transpiration rates scaled from stem sap flux measurements of fertilized (solid bars) and control (open bars) box elder trees during the 2006 and 2007 growing seasons at the experimental riparian system near Salt Lake City, UT. Error bars represent ± 1 standard error of the mean.

0.21, Table 2; Figure 6b). The lower $\delta^{13}\text{C}$ of both plots in 2007 combined with the observed monthly $\delta^{13}\text{C}$ increase in 2006 suggests that water limitations were more substantial in 2006 and progressed throughout the growing season. There was no plot*month interaction in either year (Table 2), suggesting that stream nitrogen fertilization had no impact on the relative balance between the supply and demand of CO_2 at the leaf scale.

[32] At the beginning of the experiment in 2006, leaf nitrogen per unit mass (N_{mass}) and leaf nitrogen per unit area (N_{area}) were higher in the control plot, and remained higher throughout the growing season (Table 2; Figures 6c and 6e). Conversely, N_{mass} and N_{area} of the fertilized plot increased relative to the control plot in 2007, and differences between plots disappeared (Table 2; Figures 6d and 6f). There was no significant plot*month interaction in either 2006 or 2007. Mean leaf C:N ratios were lower in the control plot

throughout the 2006 growing season (Figure 6g), while C:N ratios were similar between plots in 2007 (Figure 6h). Again, there were no significant plot*month interactions in either 2006 or 2007 (Table 2).

[33] At the onset of the experiment, stem diameter of the control trees was significantly larger than in the fertilized trees, and the estimated sapwood area was marginally higher in the control trees relative to the fertilized trees (Table 3). Differences in both stem diameter and sapwood area were maintained after the 2006 and 2007 growing seasons (Table 3), although stem diameter growth was 15% greater in fertilized trees, relative to control trees during the 2007 growing season ($P = 0.059$, data not shown). Estimates of sapwood area were combined with J_s to calculate mean daily water use for each tree during the 2006 and 2007 growing seasons. Mean daily transpiration of the control trees was 41% higher relative to the fertilized trees during the 2006 growing season ($F = 5.23$, $P = 0.0297$, Figure 7). Mean daily transpiration in 2007 remained 22% higher in the control trees relative to the fertilized trees, although differences between plots were no longer significant ($F = 1.34$, $P = 0.26$). The control and fertilized trees used, on average, 16.2 (SE = 1.5) and 11.5 (SE = 1.4) l day^{-1} , respectively throughout the 2006 growing season, and 25.5 (SE = 3.0) and 20.9 (SE = 2.7) l day^{-1} , respectively, during the 2007 growing season.

[34] Leaf area of the control trees was 36% higher than in the fertilized trees at the conclusion of the 2006 growing season ($P = 0.0238$, Table 3). Conversely, leaf area of the fertilized trees was only 17% lower than the control trees at the conclusion of the 2007 growing season, and differences between plots were no longer significant (Table 3). There were no statistical differences in either LAI or $A_1:A_s$ between plots throughout the investigation (Table 3). There were no plot differences in either canopy height or radius throughout the experiment (Table 3).

4. Discussion

[35] Increases in aquatic nitrogen over the last several decades have caused great concern for the quality and

Table 3. Mean Stem Diameter, Sapwood Area (A_s), Canopy Height, Canopy Radius, Leaf Area (A_1), LAI, and Leaf Area to Sapwood Ratio ($A_1:A_s$) of Box Elder Trees at the Beginning and Conclusion of the 2006 Growing Season and Conclusion of the 2007 Growing Season in an Experimental Riparian System in Salt Lake City, UT^a

Plot	Stem Diameter (m)	Sapwood Area (m^2)	Canopy Height (m)	Canopy Radius (m)	Leaf Area (m^2)	LAI	$A_1:A_s$
<i>2006 (Beginning)</i>							
Fertilized	0.067 (0.003)	0.026 (0.002)	3.35 (0.18)	1.13 (0.05)			
Control	0.075 (0.002)	0.032 (0.002)	3.59 (0.16)	1.24 (0.05)			
<i>P</i>	0.0276	0.06	0.66	0.18			
<i>2006 (Conclusion)</i>							
Fertilized	0.083 (0.004)	0.038 (0.003)	3.86 (0.21)	1.47 (0.06)	23.60 (2.12)	3.77 (0.50)	6360 (457)
Control	0.095 (0.003)	0.048 (0.002)	4.01 (0.15)	1.59 (0.07)	32.09 (3.21)	4.07 (0.35)	6612 (443)
<i>P</i>	0.0097	0.0193	0.66	0.18	0.0238	0.62	0.64
<i>2007 (Conclusion)</i>							
Fertilized	0.098 (0.005)	0.051 (0.004)	4.21 (0.21)	1.65 (0.06)	27.97 (2.39)	3.40 (0.32)	5645 (436)
Control	0.110 (0.003)	0.060 (0.003)	4.34 (0.18)	1.75 (0.06)	33.56 (3.97)	3.44 (0.27)	5392 (447)
<i>P</i>	0.0436	0.06	0.59	0.18	0.24	0.89	0.59

Bold data indicate significant differences at $P \leq 0.05$.

^aFertilized trees ($n = 17$) received stream water plus NH_4NO_3 , while control trees ($n = 16$) received stream water without nitrogen addition. Numbers in parenthesis are the standard errors of the means.

function of stream ecosystems [Pimental, 1993]. In the United States, for example, agriculture, industrialization and urban expansion have resulted in a doubling of nitrogen inputs into streams over the last 40 years [Howarth *et al.*, 2002]. Although negative impacts on aquatic ecosystems and water quality have been well documented [Wigington *et al.*, 1996a, 1996b; Rabalais *et al.*, 2002; Wellington and Driscoll, 2004], there is considerable uncertainty of stream nitrogen loading impacts on the terrestrial component of riparian ecosystems. This uncertainty is partially a function of large spatial and temporal variations in confounding factors such as streamflow, geomorphology, plant community structure and anthropogenic land use. The artificial riparian stream system allowed us to control for confounding variables and test for feedbacks of stream nitrogen inputs on important riparian ecosystem processes. In the present study, we focused on feedbacks of DIN on water use and productivity of a dominant riparian tree species: box elder (*A. negundo*), while highlighting potential whole ecosystem feedbacks on water and nitrogen cycling.

[36] Sap flux density increased in fertilized trees relative to trees that received no DIN amendments throughout both the 2006 and 2007 growing seasons. In 2006, the relative differences in J_s were fairly ambiguous, and increases in $J_{s,\text{Fert}}:J_{s,\text{Con}}$ were not detected until near the end of the growing season. One possible explanation for the lagged response to stream fertilization is that water may have still been limiting throughout much of the 2006 growing season. If only a small portion of the root systems were in contact with soil water in and around the stream channels, water use patterns and productivity would be limiting in what is otherwise an arid environment. In other words, the root distribution patterns may not have adjusted to the stream channel that was only constructed a few months prior to the experiment. This is supported by the lower mean daytime sap flux in 2006 compared to 2007, despite similarities in streamflow rate and volumetric soil moisture (data not shown) between years. Moreover, J_s in 2006 was not at all correlated with atmospheric vpd, as would be expected in systems where soil water is not limiting. These data suggest that the riparian trees may have required a full growing season to equilibrate with the artificial stream system.

[37] In 2007, there was a 30% ($0.4\% \text{ day}^{-1}$) increase in $J_{s,\text{Fert}}:J_{s,\text{Con}}$ from mid May through mid August. Whether these relative changes in J_s reflect scalable changes in ET and the water cycle is an open question. Changes in ET from riparian vegetation do, in some cases, impact the water cycle of riparian systems [Bosch and Hewlett, 1982; Calder, 1990; Zhang *et al.*, 2001; Huxman *et al.*, 2005; Hultine *et al.*, 2007]. In the present study, the total decline in streamflow in each plot, measured periodically as the total daily inflow minus the total daily outflow (measured by capturing the outflow of water over one minute sequences), ranged from 550–650 l per day, or about 3% of the total daily input. Total daily tree water use per plot during the 2007 growing season typically ranged from 300 to 500 l (18–31 l per tree), or about 55 to 75% of estimated stream water depletions. These values represent a fairly small component of the total water budget. However, larger and denser stands along much longer stream reaches have the potential to remove a significantly larger proportion of stream water. For

example, stream discharge at the mouth of Red Butte Canyon (directly above the growth site) declined diurnally by an average of 8% per day during the 2004 growing season, while ET rates from streamside vegetation averaged about 9% of stream discharge [Hultine *et al.*, 2007]. Dahm *et al.* [2002] estimated that riparian vegetation removed 20–33% of total estimated depletions along the Rio Grande River in New Mexico. Therefore, if ET does increase in response to stream DIN concentrations, it is plausible that large inputs of N from human activities could have unintended impacts on ecohydrological processes such as streamflow and groundwater recharge.

[38] Water transport through plants is generally proportional to growth rate and productivity [Enquist, 2002]. Consequently, the higher J_s in fertilized trees in 2007 could result in higher relative growth rates, and more leaf litter returning to the soils than in the control trees. In 2007, relative stem diameter growth was higher and leaf area increased in the fertilized trees compared to the control trees. A higher relative growth rate and higher gross primary productivity of streamside vegetation may result in a positive feedback toward greater uptake and retention of stream NO_3 . On the other hand, enhanced DIN may ultimately result in a reduction in the ratio of absorbing root area to leaf area ($A_r:A_l$) [Ewers *et al.*, 2000]. A lower $A_r:A_l$ would have a negative feedback on relative growth rates (RGR), fitness, and survival of streamside vegetation if increased DIN in stream water were met with reduced streamflow (i.e., reduced plant water availability) that often occurs in watersheds that are heavily impacted by anthropogenic land use.

[39] Dissolved inorganic nitrogen in stream water has the potential to impact the amount and quality of leaf litter that returns to the soil [Dodds *et al.*, 2004]. Enhanced LAI from fertilization would result in a larger pool of easily decomposable plant litter (i.e., leaves). Nevertheless, elevated stream DIN concentrations apparently did not result in higher LAI values in the present study. Alternatively, stream DIN could improve litter quality of streamside vegetation if leaf N content increased and/or leaf C:N decreased. In the present study, leaf N content was higher and leaf C:N was lower in the control plot compared to the fertilized plot at the onset of the experiment. However, differences in these parameters were not maintained in the second year of the study as leaf N of the fertilized plot increased and C:N decreased relative to the control plot. A reduction of C:N ratios of organic matter that enters the terrestrial system as detritus could increase rates of nitrogen mineralization [Aber and Mellilo, 1980; McClaugherty *et al.*, 1985; Dodds *et al.*, 2004]. Therefore enhanced stream DIN concentrations could result in a positive feedback of greater nitrogen availability through enhanced mineralization of organic N provided by the terrestrial system [Schade *et al.*, 2002].

[40] The proportion of plant nitrogen that is acquired from stream DIN is strongly correlated to the amount of plant-available nitrogen that is already in the ecosystem [Schade *et al.*, 2002]. Amounts of total N in study soils were low, but relatively large amounts of inorganic nitrogen could have been present from two potential sources: mineralization from organic matter in the soil or inorganic N from atmospheric deposition. Given that total N contents were low and the riparian system was built on fill material without a noticeable organic layer, it is unlikely that the system was

high in available N from organic material. Likewise, concentrations of atmospheric N in the western United States are generally low [Fenn *et al.*, 2003]. However, some aquatic and terrestrial plant and microbial communities have been significantly altered by N deposition [Williams *et al.*, 1996; Fenn and Poth, 1999; Egerton-Warburton and Allen, 2000], especially near expanding metropolitan areas such as Salt Lake City [Fenn *et al.*, 2003]. If plant available N was enhanced by atmospheric deposition, then tree uptake of stream N may have been constrained relative to habitats that occur in non-urbanized locations.

[41] Leaf $\delta^{15}\text{N}$ values strongly suggest that the box elder trees were in fact acquiring DIN from stream water. In year two (2007) of the present study, we added a ^{15}N tracer to the stream DIN to determine if the box elder trees were acquiring DIN from the stream. The isotope tracer raised the $\delta^{15}\text{N}$ of stream fertilizer from -0.2‰ to approximately 200‰ , while leaf $\delta^{15}\text{N}$ showed a significant, but much smaller increase of about 2‰ relative to trees in the control plot. The small increase in leaf $\delta^{15}\text{N}$, relative to the tracer, makes it uncertain how much stream DIN contributed to the nitrogen balance of the trees. One consideration is that the system may have been saturated with nitrogen from stream fertilization during the 2006 growing season. Another important consideration is that there may have been discrimination against the heavier ^{15}N during uptake of NO_3 . In environments where external pools of NO_3 are high relative to demand, discrimination may reach 20‰ during plant uptake [Evans, 2001]. Moreover, fractionation of N may also occur during the uptake and translocation of N from mycorrhizal symbionts [Högberg, 1997]. Given the observed relative change in water use patterns between plots, it appears likely that the elevated stream DIN did provide a measurable source of nitrogen to the box elder trees.

5. Conclusions and Implications

[42] Although many investigations have addressed ecosystem responses to fertilization, very few investigations have focused on riparian ecosystem responses to elevated stream DIN concentrations. Information on ecosystem-scale responses to stream N loading is largely absent, partially because of the difficulty in controlling for confounding variables in time and space. The construction and utilization of artificial riparian stream systems, such as the one described here, allows for relatively large-scale manipulations, while reducing large spatial and temporal variation (although pseudo-replication is still an inevitable consequence of single-plot manipulations). In the present study, increases in tree sap flux rates and changes in leaf chemistry were related to enhanced DIN. Increases in above-ground RGR and leaf area were also detected, although these changes were small and may require longer study periods than two growing seasons to monitor. Results from this investigation indicate that substantial inputs of inorganic nitrogen into stream systems may have significant feedbacks on the function of streamside vegetation. Potential feedbacks include higher rates of water loss, enhanced primary productivity, greater relative allocation of resources to above-ground tissues (i.e., reduced root surface area to leaf area ratios), and reduced drought tolerance. Functional plant responses to elevated stream nitrogen could have

measurable impacts on several ecohydrological processes including among many others: evapotranspiration, streamflow, net ecosystem productivity, and nitrogen cycling in both terrestrial and aquatic environments.

[43] As global inputs of anthropogenic DIN into stream systems continue to escalate, an improved understanding of ecosystem-scale feedbacks of stream N loading will progressively take on an added importance. The construction and utilization of experimental riparian stream systems provide researchers with the framework to investigate these important issues over large temporal scales. Currently, our research at the experimental stream system described here is focusing on stream DIN impacts on whole ecosystem water balance, soil respiration and plant respiration, whole plant carbon balance, plant community ecology, and litter quality. Future research will address combined impacts of global change induced alteration of streamflow and stream DIN concentrations on whole ecosystem water, carbon, and nitrogen cycling.

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References

- Aber, J. D., and J. M. Melillo (1980), Litter decomposition: Measuring relative contributions of organic matter and nitrogen to forest soils, *Can. J. Bot.*, *58*, 416–421.
- Belnap, J., J. R. Welter, N. B. Grimm, N. Barger, and J. A. Ludwig (2005), Linkages between microbial and hydrologic processes in arid and semi-arid watersheds, *Ecology*, *86*, 298–307.
- Bosch, J. H., and J. D. Hewlett (1982), A review of catchment experiments to determine the effect of vegetation changes and water yield and evapotranspiration, *J. Hydrol.*, *55*, 3–23.
- Calder, I. R. (1990), *Evaporation in the Uplands*, John Wiley, New York.
- Dahm, C. N., J. R. Cleverly, J. E. Allred Coonrod, J. R. Thibault, D. E. McDonnell, and D. J. Gilroy (2002), Evapotranspiration at the land/water interface in a semi-arid drainage basin, *Freshwater Biol.*, *47*, 831–843.
- Dodds, W. K., et al. (2004), Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams, *Oecologia*, *140*, 458–467.
- Egerton-Warburton, L. M., and E. B. Allen (2000), Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient, *Ecol. Appl.*, *10*, 484–496.
- Ehleringer, J. R. (1991), $^{13}\text{C}/^{12}\text{C}$ fractionation and its utility in terrestrial plant studies, in *Carbon Isotope Techniques*, edited by D. C. Coleman and B. Fry, pp. 187–200, Academic, New York.
- Enquist, B. J. (2002), Universal scaling in tree and vascular plant allometry: Toward a general quantitative theory linking plant form and function from cells to ecosystems, *Tree Physiol.*, *22*, 1045–1064.
- Evans, R. D. (2001), Physiological mechanisms influencing plant nitrogen isotope composition, *Trends Plant Sci.*, *6*, 121–126.
- Ewers, B. E., R. Oren, T. J. Albaugh, and P. M. Dougherty (1999), Carry-over effects of water and nutrient supply on water use of *Pinus taeda*, *Ecol. Appl.*, *9*, 513–525.
- Ewers, B. E., R. Oren, and J. S. Sperry (2000), Influence of nutrient versus water supply on hydraulic architecture and water balance in *Pinus taeda*, *Plant Cell Environ.*, *23*, 1055–1066.
- Ewers, B. E., R. Oren, N. Phillips, M. Strömberg, and S. Linder (2001), Mean canopy stomatal conductance responses to water and nutrient availabilities in *Picea abies* and *Pinus taeda*, *Tree Physiol.*, *21*, 841–850.
- Fenn, M. E., and M. A. Poth (1999), Temporal and spatial trends in stream-water nitrate concentrations in the San Bernardino Mountains, southern California, *J. Environ. Qual.*, *28*, 822–836.
- Fenn, M. E., et al. (2003), Ecological effects of nitrogen deposition in the western United States, *BioScience*, *53*, 404–420.
- Ferreira, V., V. Gulis, and M. A. S. Graca (2006), Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates, *Oecologia*, *149*, 718–729.

- Giardina, C. P., M. G. Ryan, D. Binkley, and J. H. Fownes (2003), Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest, *Global Change Biol.*, *9*, 1438–1450.
- Gower, S. T., K. A. Vogt, and C. C. Grier (1992), Carbon dynamics of rocky mountain Douglas-fir influences of water and nutrient availability, *Ecol. Monogr.*, *62*, 43–65.
- Granier, A. (1987), Evaluation of transpiration in a Douglas fir stand by means of sap flow measurements, *Tree Physiol.*, *3*, 309–320.
- Hacke, U. G., J. S. Sperry, B. E. Ewers, D. S. Ellsworth, K. V. R. Schäfer, and R. Oren (2000), Influence of soil porosity, on water use in *Pinus taeda*, *Oecologia*, *124*, 495–505.
- Högberg, P. (1997), Tansley review no. 95. ¹⁵N natural abundance in soil-plant systems, *New Phytol.*, *137*, 179–203.
- Howarth, R. W., E. W. Boyer, W. J. Pabich, and J. N. Galloway (2002), Nitrogen in the United States from 1961 to 2000 and potential future trends, *Ambio*, *31*, 88–96.
- Hubbard, R. M., M. G. Ryan, C. P. Giardina, and H. Barnard (2004), The effect of fertilization on sap flux and canopy conductance in a *Eucalyptus saligna* experimental forest, *Global Change Biol.*, *10*, 427–436.
- Hultine, K. R., S. E. Bush, A. G. West, and J. R. Ehleringer (2007), The effect of gender on sap flux-scaled transpiration in a dominant riparian tree species: Box elder (*Acer negundo*), *J. Geophys. Res.*, *112*, G03S06, doi:10.1029/2006JG000232.
- Huxman, T. E., B. P. Wilcox, D. D. Breashears, R. L. Scott, K. A. Snyder, E. E. Small, K. Hultine, W. T. Pockman, and R. B. Jackson (2005), Ecohydrological implications of woody plant encroachment, *Ecology*, *86*, 308–319.
- Jarvis, P. G., and K. G. McNaughton (1986), Stomatal control of transpiration: Scaling up from leaf to region, *Adv. Ecol. Res.*, *15*, 1–49.
- Jordan, T. E., and D. E. Weller (1996), Human contributions to the terrestrial nitrogen flux, *BioScience*, *46*, 655–663.
- Lowrance, R. R. (1998), Riparian forest ecosystems as filters for nonpoint-source pollution, in *Successes, Limitations, and Frontiers in Ecosystem Science*, edited by M. L. Pace and P. M. Groffman, pp. 113–141, Springer-Verlag, New York.
- Mack, M. C., E. A. G. Schuur, M. S. Bret-Harte, G. R. Shaver, and F. S. Chapin (2004), Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization, *Nature*, *431*, 440–443.
- Martin, P. (1989), The significance of radiative coupling between vegetation and the atmosphere, *Agric. For. Meteorol.*, *49*, 45–53.
- McClaugherty, C. A., J. Paster, and J. D. Aber (1985), Forest litter decomposition in relation to soil nitrogen dynamics and litter quality, *Ecology*, *66*, 266–275.
- Meybeck, M. (1982), Carbon, nitrogen, and phosphorus transport by world rivers, *Am. J. Sci.*, *282*, 401–450.
- Nydahl, F. (1976), On the optimum conditions for the reduction of nitrate by cadmium, *Talanta*, *23*, 349.
- Parsons, T. R., Y. Maita, and C. M. Lalli (1984), *A Manual of Chemical and Biological Methods for Seawater Analysis*, Oxford Univ. Press, Oxford, New York.
- Phillips, N., J. Bergh, R. Oren, and S. Linder (2001), Effects of nutrition and soil water availability on water use in a Norway spruce stand, *Tree Physiol.*, *21*, 851–860.
- Pimental, D. (1993), *World Soil Erosion and Conservation*, Cambridge Press, Cambridge, Mass.
- Potvin, C., M. J. Lechowicz, and S. Tardiff (1990), The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures, *Ecology*, *71*, 1389–1400.
- Rabalais, N. N., R. E. Turner, and D. Scavia (2002), Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River, *BioScience*, *52*, 129–141.
- Sala, A., S. D. Smith, and D. A. Devitt (1996), Water use by *Tamarix ramosissima* and associated phreatophytes in a Mojave Desert floodplain, *Ecol. Appl.*, *6*, 888–898.
- Schade, J. D., E. Marti, J. R. Welter, S. G. Fisher, and N. B. Grimm (2002), Sources of nitrogen to the riparian zone of a desert stream: Implications for riparian vegetation and nitrogen retention, *Ecosystems*, *5*, 68–79.
- Schuur, E. A. G., and P. A. Matson (2001), Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest, *Oecologia*, *128*, 431–442.
- Sperry, J. S., F. R. Adler, G. S. Campbell, and J. P. Comstock (1998), Limitation of plant water use by rhizosphere and xylem conductance: Results from a model, *Plant Cell Environ.*, *21*, 347–359.
- Wellington, B. I., and C. T. Driscoll (2004), The episodic acidification of a stream with elevated concentrations of dissolved organic carbon, *Hydrol. Processes*, *18*, 2663–2680.
- Wigington, P. J., J. P. Baker, D. R. DeWalle, W. A. Kretser, P. S. Murdoch, H. A. Simonin, J. Van Sickle, M. K. McDowell, D. V. Peck, and W. R. Barchet (1996a), Episodic acidification of small streams in the northeastern United States: Episodic response project, *Ecol. Appl.*, *6*, 374–388.
- Wigington, P. J., D. R. DeWalle, P. S. Murdoch, W. A. Kretser, H. A. Simonin, J. VanSickle, and J. P. Baker (1996b), Episodic acidification of small streams in the northeastern United States: Ionic controls and episodes, *Ecol. Appl.*, *6*, 389–407.
- Williams, M. W., J. S. Baron, N. Caine, R. Sommerfield, and R. Sanford (1996), Nitrogen saturation in the Rocky Mountains, *Environ. Sci. Technol.*, *30*, 640–646.
- Yamada, T., S. D. Logsdon, M. D. Tomer, and M. R. Burkart (2007), Groundwater nitrate following the installation of a vegetated riparian buffer, *Sci. Total Environ.*, *385*, 297–309.
- Zhang, L., R. W. Dawes, and G. R. Walker (2001), Response of mean annual evapotranspiration to vegetation changes at catchment scale, *Water Resour. Res.*, *37*, 701–708.

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