

Isotopic Evidence of the Effects of Herbivory and Landscape Position on Plant Nitrogen Sources in a Riparian Ecosystem

By

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Abstract. During the last few decades, changes in ungulate grazing and surface water hydrology have occurred in elk winter ranges in Rocky Mountain National Park (RMNP). These changes may be influencing the biogeochemistry and vegetation structure of these habitats, as evidenced by the decline in willow (*Salix* spp.) communities. Previous studies in riparian areas of RMNP demonstrated that sedges (*Carex* spp.) and willow utilize different proportions of groundwater vs. rainwater across different landscape positions in those habitats. However, whether these two plant growth forms differ in their sources of N has remained unknown. We conducted field studies in elk winter ranges in RMNP during the growing seasons of 1997 and 1998. In these studies, we utilized ¹⁵N natural abundance and non-isotopic techniques to identify the N sources of willow and sedge under different herbivory treatments and landscape positions. Based on the isotopic evidence, we found that sedge plants seemed to acquire smaller proportions of groundwater N in upper landscape positions in comparison to the woody willow species, suggesting that the deeper rooting characteristics of willow may allow these plants to access more groundwater N. However, grazed willow plants in upper landscape positions seemed to acquire less groundwater N as compared to willow plants protected from herbivory. Therefore, it appears that herbivory by elk and the shifts in landscape hydrology, caused by reductions in beaver activity and a warming and drying trend, could have an interacting effect on willow by increasing the frequency of drought stress and possibly reducing the availability of nitrogen. The combination of these factors could explain, in part, the decline of willow communities across riparian habitats in Rocky Mountain National Park.

Keywords: ¹⁵N natural abundance, beaver, *Carex*, elk, Rocky Mountain National Park, *Salix*, willow.

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Introduction

Changes in ungulate grazing and hydrology have occurred in the winter ranges of elk in Rocky Mountain National Park (RMNP) during the last few decades. These changes may be influencing the biogeochemistry and vegetation structure of these systems as evidenced by reductions in willow (*Salix* spp.) growth (Singer et al. 1998). Elk numbers have increased 3-fold (Singer et al. 1998) over the last 30 years, and currently a high density of elk (*Cervus elaphus*) graze these systems in winter. Changes in the hydrology of these systems are evident from aerial photographs between 1937 and 1996, which indicate that rivers are now less braided and there is less surface water than in the beginning of this century (Peinetti et al., this volume). These changes in hydrology are probably a result of the observed declines in beaver (*Castor canadensis*) activity (Stevens and Christianson 1980), which may change the biogeochemical cycles by altering the availability of stream and groundwater (Naiman et al. 1994; Terwilliger and Pastor 1999).

Based on $\delta^{18}\text{O}$ measurements, Alstad et al. (1999) found that sedge (*Carex* spp.) and willow in the elk winter ranges of RMNP utilize water from different sources. Willow appears to rely on groundwater (80% of total water uptake), which is recharged by streamwater throughout the floodplains. In addition, water sources of willow seemed to be independent of landscape position and consistent throughout the growing season. In contrast, sedges utilized mostly groundwater early in the season but seemed to take up increasing amounts of rainwater (up to 50%) towards the end of the season, especially when located in upper landscape positions. These differences in water source between the two plant functional groups may result from differences in rooting characteristics, but whether these two plant growth forms differ in their sources of nitrogen (N) is unknown. Based on the $\delta^{18}\text{O}$ measurements of Alstad et al. (1999), we anticipated that willows may be relying more strongly on groundwater N in comparison to sedges, especially in the case of plants located in upper landscape positions.

The mineral nutrition of plants in native habitats influences a suite of physiological and ecological processes such as carbon exchange, stress resistance, and competitive interactions (Chapin 1980; Welker et al. 1987, 1991; Nadelhoffer et al. 1999). Of the nutrients that may influence and limit plant growth, nitrogen is usually the most limiting element in terrestrial ecosystems (Aber et al. 1989; Nadelhoffer et al. 1999).

Identifying the sources of plant nitrogen in terrestrial ecosystems cannot be easily done, but stable isotope techniques can be employed (Garten 1993; Michelsen et al. 1996; Nadelhoffer et al. 1996) especially under controlled experimentation and in combination with non-isotopic techniques (Handley and Scrimgeour 1997). Plants may acquire two forms of nitrogen mineralized from soil organic matter (NH_4^+ and NO_3^-) that may have different $\delta^{15}\text{N}$ values due to kinetic effects on isotope discrimination during organic matter decomposition (Shearer and Kohl 1986; Evans et al. 1996). In addition, a few studies have reported the uptake of organic forms of N from soil by some plants in arctic ecosystems (Chapin et al. 1993; Kielland 1994; Nasholm et al. 1998), but the relevance of the uptake of soil organic N forms by plants in other systems, including our study sites, is unknown, and still needs further investigation. In riparian habitats, soil inorganic N is not the only N source, as ground and stream water may contain inorganic N. Therefore, in riparian ecosystems, if the $\delta^{15}\text{N}$ signature of soil and water N sources differ, stable isotope techniques could be useful in combination with other approaches to characterize the patterns of plant N uptake (Handley and Raven 1992).

The primary questions asked by this study were:

- (1) Are there differences in the N uptake patterns of willow and sedge in different landscape positions?
- (2) How does winter elk herbivory affect the patterns of N uptake by willow and sedge in different landscape positions? Based on these questions, we hypothesized that: (a) sedge relies mostly on soil inorganic N in upper landscape positions, and on both soil inorganic N and groundwater N in lower landscape positions, following the patterns of water uptake; (b) regardless of landscape position, willow plants protected from grazing utilize a mixture of both groundwater and soil inorganic N because these plants, independently of landscape position, have access to the groundwater table; and (c) willow plants under elk grazing in upper landscape positions take up less groundwater N because grazing by elk may lead to a reduction in belowground carbon (C) allocation and limit the ability of these plants to reach the groundwater.

In order to test our hypotheses, we conducted field studies on the elk winter ranges in RMNP during the 1997 and 1998 growing seasons. In these studies, we utilized both ^{15}N natural abundance and non-isotopic biogeochemical techniques to identify the nitrogen sources of willow and sedge plants under herbivory treatments and different landscape positions.

Methods

Study Sites

Our study sites were located in two riparian ecosystems on the northeastern side of Rocky Mountain National Park: Moraine Park (Big Thompson River watershed, elevation 2,481 m) and Horseshoe Park (Fall River watershed, elevation 2,598 m). The two watersheds are within 5 km of each other and have perennial alpine snowfields at their headwaters (Baron 1992). Mean annual precipitation for the sites is 41 cm (Singer et al. 1998) and peak stream flow usually occurs in early to mid-June (USDA 1995, 1996). The 30-year average temperature for the adjacent Estes Valley ranges from 9 to 17°C during the 5-month growing season of May through September (Alstad et al. 1999). The study area consists of open grasslands and wet meadows dominated by sedges (*Carex* spp.) and willow (*Salix monticola*, *S. geyeriana*, and *S. planifolia*), with some birch (*Betula* spp.) (Singer et al. 1998). Among the *Salix* species, *S. monticola* is dominant in most areas and, for this reason, was selected as the target species for this study.

Grazing Treatments

Twelve 30 m x 46 m enclosures were erected to eliminate elk browsing (ungrazed plots) at randomly chosen sites within both riparian zones (six sites each in Horseshoe Park and Moraine Park) between August and November of 1994. Near each enclosure, a 30 m x 46 m plot area was marked off as a paired plot that was grazed by elk in winter (grazed plots). In each enclosure and associated grazed plot, an average of five shallow (0.5 m to 2 m) wells were installed using PVC pipes in the fall of 1994 to monitor groundwater levels and chemistry.

Design of Preliminary Experiment in 1997

Eight of the 12 sites in Moraine and Horseshoe Parks were selected (four sites in each park), and two willow plants were selected within each grazing treatment (grazed and ungrazed) for physiological and biogeochemical measurements. One representative plant was chosen in close proximity to streams (streamside) and another in areas distant from streams (upper landscape, which were at least 10 m in a horizontal direction from streams and approximately 0.4 to 0.6 m higher in elevation than streamside plants), resulting in a total of 32 selected plants and four experimental replications in each park. Sedge plots (1 m x 1 m) were

marked next to each selected willow plant (within 2 m of distance). During August 1997, samples from non-photosynthetic tissue were collected from current year growth of willow and sedge plants, dried at 60°C for 72 hours, ground in a ball mill to pass a 0.5 mm sieve, and stored until analysis. Concentrations of carbon and nitrogen in plant tissue were determined using a LECO CHN Analyzer.

Surface water samples were collected with plastic vials from the Big Thompson and Fall Rivers in June, July, and August. Samples were collected at different dates from each river, combined in a single sample, and kept frozen until isotopic and chemical analyses.

In July 1997, 4-week in situ field soil incubations using aluminum cores (5 cm in diameter, 15 cm in length) were conducted according to the methodology described by Kolberg et al. (1997). Incubation cores (n = 5) were placed next to plants located at both streamside and upper landscape positions. At the end of the incubation period, the soil cores were collected, placed in plastic bags, kept refrigerated in coolers, taken to the laboratory, and kept refrigerated. Within two days of collection, the total soil weight of each sample was recorded, sub-samples (25 g) were extracted with 50 ml of 2 M KCl for 1 hour, filtered, and the extracts kept frozen until analysis. The total inorganic N (NH_4^+ plus NO_3^-) in river water samples and soil KCl extracts were diffused into 5 mm acidified filter paper disks according to the methodology described by Khan et al. (1998).

Experimental Design and Sampling in 1998

After observing a sharp contrast in the isotopic signatures of N sources and plants during the growing season of 1997, we established a different experimental design and conducted a more detailed study during the growing season of 1998. In this study, a total of 50 willow plants (23 and 27 in grazed and ungrazed plots, respectively) were selected from the 12 research sites of Moraine Park and Horseshoe Park. All the selected plants were located within 3 m from a well, in order to allow groundwater sampling and the determination of the exact water table depth next to each selected plant. Plants selected within the same site and grazing treatment were located at positions with different water table depths. Next to each willow plant (within 1 m), an associated sedge plot (0.5 m x 0.5 m) was marked for the purpose of collecting sedge tissue samples. Willow and sedge tissue samples (current annual growth [CAG]) were collected

in both early July and early September, dried at 60°C for 72 hours, and then ground in a ball mill to pass a 0.5 mm sieve. Concentrations of carbon and nitrogen in plant tissue were determined using a LECO CHN Analyzer.

River water samples were collected from both the Big Thompson and Fall Rivers in mid-July, mid-August, and mid-September by immersing a 3.8 L container at different points along the river surface in the area of the experimental plots. The water samples were kept refrigerated in coolers until the inorganic N was diffused into 5 mm acidified filter paper disks as described by Khan et al. (1998).

Groundwater depth was monitored throughout the season by measuring the water levels in the wells associated with the plants. Groundwater samples (3.8 L) were collected in early August in each well using a small manual pump. A sub-sample (20 ml) from each groundwater and river water sample was placed into scintillation vials and frozen for the determination of groundwater N concentration using an Alpchem automated spectrophotometer. The remaining volume of each groundwater and river water sample was passed through a plastic column (20 cm long, 2 cm in diameter) filled with equal amounts of cation and anion exchange resins (US Filter, Pittsburgh, Pennsylvania). After all the water had gone through the column, the resin within each column was placed in a 250 ml Erlenmeyer flask and sequentially shaken for 15 minutes with five 30-ml aliquots of 2 M KCl as recommended by Kolberg et al. (1997). After each extraction, the extract was drained from the flask by placing a patch of nylon cloth at the mouth of the flask, and the five 30 ml aliquots from each sample were poured into a 200 ml plastic container and kept frozen until analysis. Before isotopic analysis, the N in river water and groundwater samples was diffused into 5 mm acidified filter paper disks, according to the methodology described by Khan et al. (1998).

In early August, next to each willow plant and sedge plot, three soil cores were collected (2.5 cm in diameter) to the depth of the groundwater. Each core was subdivided in 15 cm intervals up to a depth of 60 cm and 30 cm intervals up to a depth of 120 cm, depending on the depth of the water table. The soil from all three cores was combined by depth, placed in plastic bags, transported to the laboratory, air-dried, passed through a 2 mm sieve, and ground in a ball mill to pass a 0.25 mm sieve. Concentrations of total soil C and N were determined using a LECO CHN Analyzer.

Within and adjacent to the 12 exclosures, field soil incubations using aluminum cores (5 cm in diameter, 15 cm deep) were installed next to 19 willow plants and

within the sedge plots, following the same procedure as described above for the 1997 growing season (Kolberg et al. 1997). For isotopic analysis, the inorganic N in the KCl extracts was diffused into 5 mm acidified filter paper disks as described by Khan et al. (1998).

Isotopic Analyses

The isotopic signatures of plant tissue, total soil N, and diffusion disks from water samples and extracts were determined using a Carlo-Erba NA 1500 Series 2 Carbon and Nitrogen Analyzer attached to a VG-Optima mass spectrometer at the Natural Resources Ecology Laboratory, Colorado State University. Natural ^{15}N abundance is expressed as delta units (d), which denotes parts per thousand deviations ($\%$), from the ratio $^{15}\text{N}:^{14}\text{N}$ in atmospheric N_2 ,

$$\delta^{15}\text{N} = [({}^{15/14}\text{R}_{\text{sample}} - {}^{15/14}\text{R}_{\text{standard}}) / {}^{15/14}\text{R}_{\text{standard}}] \times 10^3$$

where R is the isotopic ratio and the standard is N_2 of air, which has a δ value of 0‰ (Handley and Scrimgeour 1997; Hogberg 1997).

Statistical Analyses

Statistical analyses were performed using the SAS Statistical Package (SAS 1995, Version 6.12, SAS Institute Inc., Cary, NC). During the 1997 growing season, plant tissue $\delta^{15}\text{N}$ was analyzed using a split-plot design with grazing treatments as the main factor and landscape positions as sub-plots. During the 1998 growing season, correlation and regression analyses were used to test our *a priori* hypotheses regarding the relationships between the $\delta^{15}\text{N}$ values of plant, soil or water, and landscape position.

Results

Preliminary Experiment – 1997 Growing Season

The $\delta^{15}\text{N}$ values of soil inorganic N in the experimental plots of Moraine and Horseshoe Parks averaged -6.14% (± 0.51) and were significantly ($P = 0.003$) more depleted than river water $\delta^{15}\text{N}$ values from Big Thompson and Fall Rivers, which averaged -1.00% (± 0.52). The sharp contrast between $\delta^{15}\text{N}$ values

of potential plant N sources in our sites is very relevant, as it corresponds to the differences in plant tissue $\delta^{15}\text{N}$ values in different landscape positions.

Tissue $\delta^{15}\text{N}$ values of sedge plants ranged from -0.6 to +3.2%, while for willow plants these values ranged from -4.3 to +2.2%. There were no significant effects of elk herbivory on plant tissue $\delta^{15}\text{N}$ values, but landscape position had a significant effect on sedge tissue $\delta^{15}\text{N}$ (Table 1). Sedge plants located in upper landscape positions had $\delta^{15}\text{N}$ values similar to the $\delta^{15}\text{N}$ values of soil inorganic N, while the $\delta^{15}\text{N}$ values of sedge plants located adjacent to streams were significantly ($P < 0.05$) more enriched. To the contrary, willow tissue $\delta^{15}\text{N}$ was not affected by landscape position and was consistently near the value of stream water $\delta^{15}\text{N}$.

1998 Growing Season

The patterns of $\delta^{15}\text{N}$ values of willow and sedge leaf tissue in 1998 were similar to 1997. Sedge tissue $\delta^{15}\text{N}$ was significantly influenced by landscape position (shallow water table depth is associated with streamside locations and deeper water table depth is associated with upland locations), during both July and September (Fig. 1). In contrast, willow $\delta^{15}\text{N}$ was not significantly affected by landscape position (water table depth), with the exception of grazed plants in July (Fig. 1). For both sedge and willow, there were no significant differences between the regression lines of the two grazing treatments in both sampling dates.

The data from our non-isotopic measurements yielded no significant differences between treatments.

Throughout the growing season, there were no significant correlations between plant tissue total nitrogen content and water table depth or total soil N. Willow tissue nitrogen concentration (% dry weight) was significantly higher ($P < 0.05$) in grazed than in ungrazed plots in both sampling dates, averaging 2.72% and 2.27% in July, and decreasing to 2.23% and 2.06% in September, in grazed and ungrazed plots, respectively. Sedge tissue total nitrogen content was not significantly affected by elk grazing in the two sampling dates, and averaged 1.91% and 1.87% in July and 1.61% and 1.48% in September for grazed and ungrazed plants, respectively.

Total soil $\delta^{15}\text{N}$ (0 to 15 cm) ranged from -2.8% to +8.0%, and was significantly more depleted in upper landscape positions (Fig. 2a). Soil inorganic nitrogen $\delta^{15}\text{N}$ (0 to 15 cm) ranged from -10.4% to +5.4%, and also presented a significant positive correlation with water table depth, being significantly more depleted in upper landscape positions (Fig. 2b). A significant correlation ($P < 0.001$; $r = 0.73$) was observed between $\delta^{15}\text{N}$ values of inorganic N and that of total soil N in the upper soil horizon and, on average, $\delta^{15}\text{N}$ of inorganic soil N was 2.7% more depleted in relation to total soil $\delta^{15}\text{N}$. Because field soil incubations were performed only in ungrazed plots, values of $\delta^{15}\text{N}$ of inorganic soil N in grazed plots were estimated with a linear equation, $Y = -3.71 + 1.77 X$; $r^2 = 0.53$, where Y is estimated $\delta^{15}\text{N}$ of inorganic soil N and X is $\delta^{15}\text{N}$ of total soil N. Groundwater $\delta^{15}\text{N}$ correlated negatively to water table depth in ungrazed plots (Fig. 2c), but no significant correlation ($P = 0.164$) was observed in the grazed plots. River water $\delta^{15}\text{N}$ ranged from +0.58% to +2.93%, and averaged +1.68% (± 0.94).

Table 1. $\delta^{15}\text{N}$ values of willow and sedge tissue in upper landscape and streamside positions of Horseshoe Park and Moraine Park during August 1997. Values represent means ($n = 8$) followed by the standard error between parentheses. Means followed by different letters are significantly different at $P < 0.05$.

Landscape position	Plant tissue $\delta^{15}\text{N}$ (%)	
	Sedge	Willow
Horseshoe Park		
Upper landscape	-6.10 (2.34) ^a	-1.87 (0.44) ^a
Streamside	-0.74 (0.68) ^b	-1.82 (0.27) ^a
Moraine Park		
Upper landscape	-10.84 (1.45) ^a	0.03 (0.41) ^a
Streamside	-2.28 (3.10) ^b	-0.41 (0.65) ^a

^{a,b}Different letters denote statistical differences at $P < 0.05$.

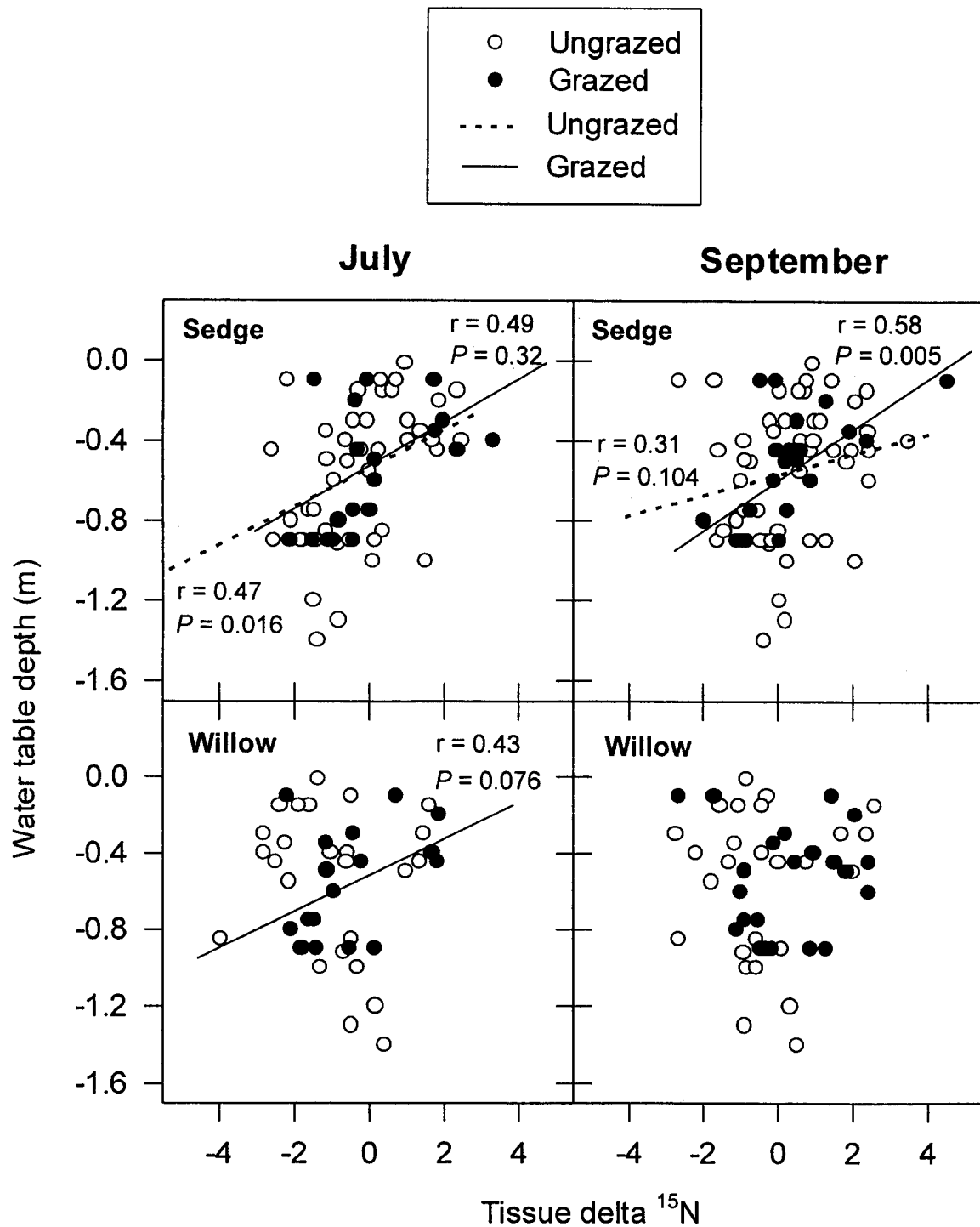


Fig. 1. Patterns of $\delta^{15}\text{N}$ values of grazed and ungrazed willow and sedge leaf tissue as related to water table depth (m) in willow communities on elk winter range of Rocky Mountain National Park, Colorado, 1998.

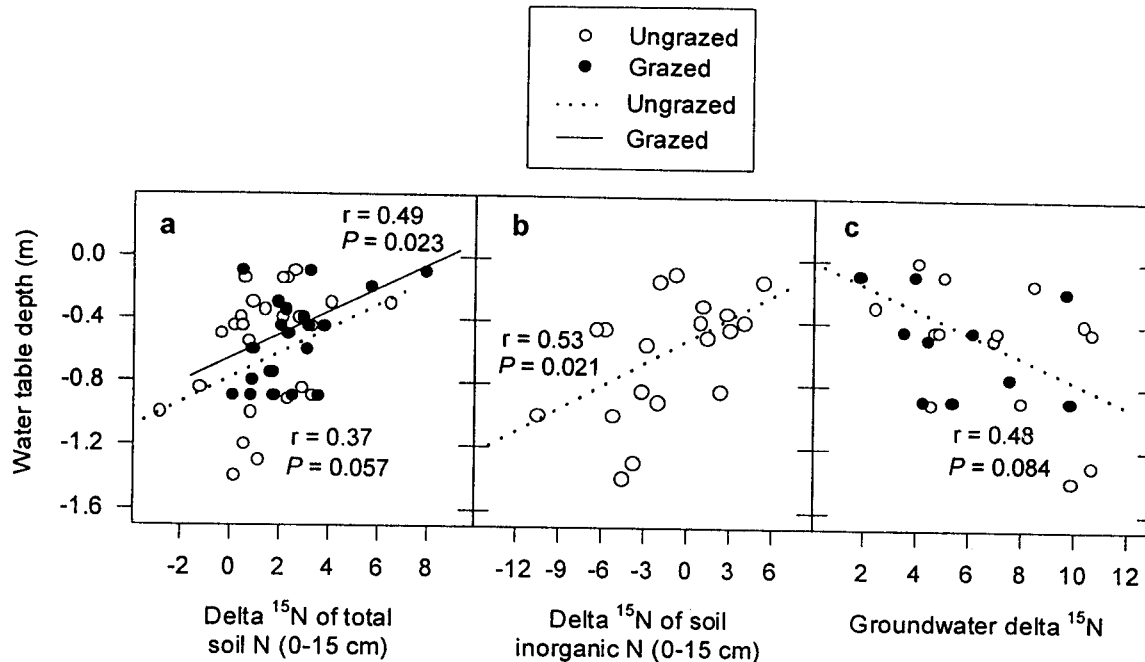


Fig. 2. Water table depth as related to $\delta^{15}\text{N}$ values of soil N (a), soil inorganic N (b), and groundwater (c) in willow communities on elk winter range of Rocky Mountain National Park, Colorado, 1998.

Sedge tissue $\delta^{15}\text{N}$ was significantly correlated to total soil $\delta^{15}\text{N}$ only in the case of grazed plants collected in September, but willow tissue $\delta^{15}\text{N}$ values of grazed and ungrazed plants were correlated to total soil $\delta^{15}\text{N}$ in both sampling dates (Fig. 3). The regression lines of the relationships between total soil $\delta^{15}\text{N}$ and tissue $\delta^{15}\text{N}$ from grazed and ungrazed sedge plants were significantly different ($P < 0.05$) in September, which suggests that grazed sedge plants may rely relatively more on soil N than groundwater N in comparison to ungrazed sedge plants and that the reliance on soil N may increase over the course of the summer (Fig. 3).

No significant correlations were observed between sedge tissue $\delta^{15}\text{N}$ and groundwater $\delta^{15}\text{N}$, regardless of grazing regime or sampling date (Fig. 4). In contrast, there were significant correlations between groundwater $\delta^{15}\text{N}$ and tissue $\delta^{15}\text{N}$ of ungrazed willow plants in both July and September (Fig. 4). Interestingly, no significant correlations were observed in the case of grazed willow plants (Fig. 4), which suggests grazed willow plants may use less groundwater N. Tissue $\delta^{15}\text{N}$ values of sedge plants

ranged from -2.6 to +4.53%, while for willow plants these values ranged from -4.0 to +2.6%. On average, $\delta^{15}\text{N}$ values of willow tissue were 2.4 and 6.9% more depleted than soil $\delta^{15}\text{N}$ and groundwater $\delta^{15}\text{N}$, respectively. Similarly, $\delta^{15}\text{N}$ values of sedge tissue were 1.8% and 6.7% more depleted than soil $\delta^{15}\text{N}$ and groundwater $\delta^{15}\text{N}$, respectively.

Discussion

The two potential inorganic N sources for riparian plants, being either from soil or from groundwater, differed in their $\delta^{15}\text{N}$ values by an average of 5% during the growing season of 1997. This difference is an important part of our study as it corresponds to the differences in the $\delta^{15}\text{N}$ values of plant tissue under divergent experimental treatments. The range of plant tissue $\delta^{15}\text{N}$ values observed in our study is consistent with values reported for plant tissue in terrestrial ecosystems, which usually vary between -5 to +2%, but

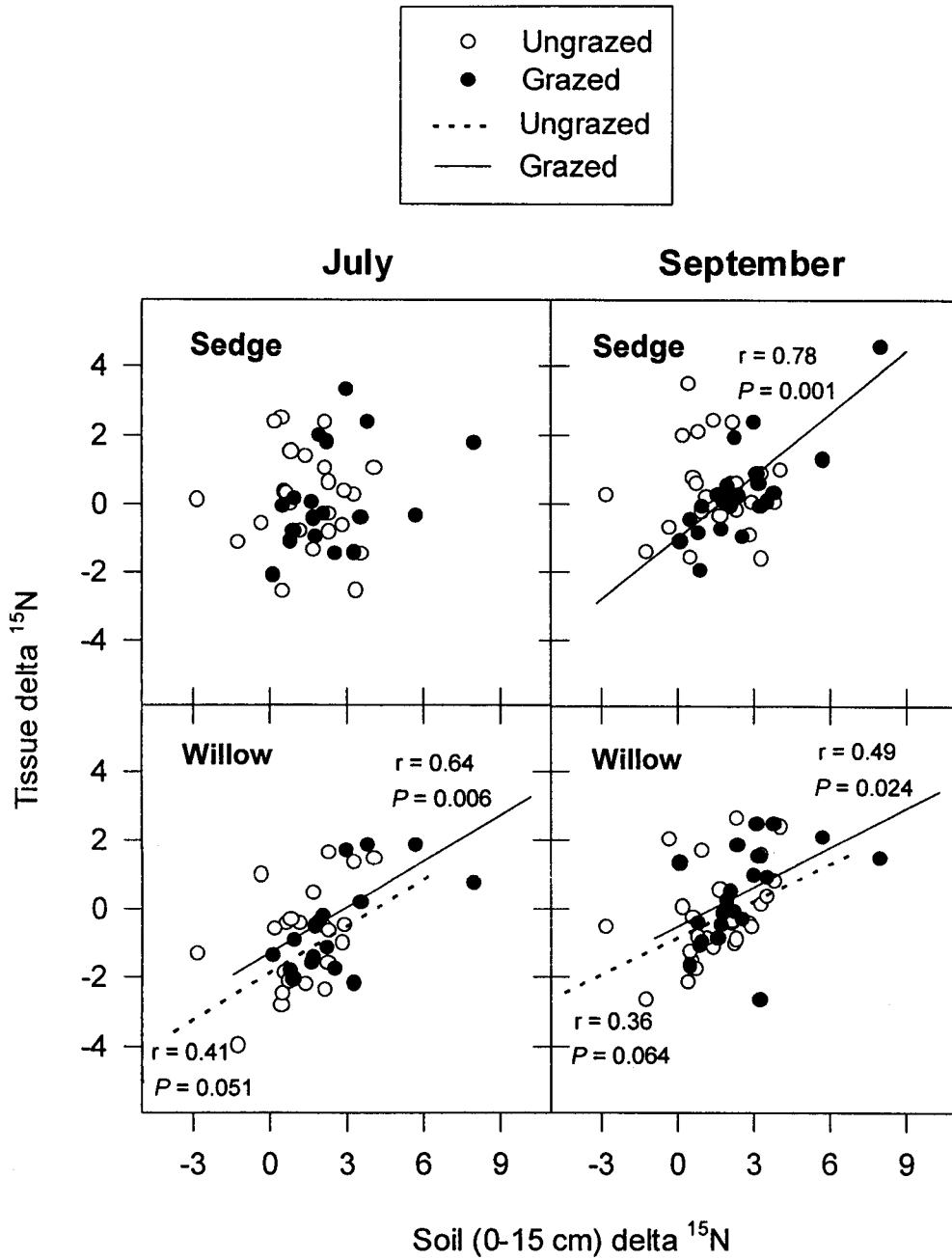


Fig. 3. Correlations between $\delta^{15}\text{N}$ values of total soil N (0–15 cm) and sedge (a and b) and willow (c and d) tissue in July and September of 1998 in grazed and ungrazed plots of Moraine and Horseshoe Parks.

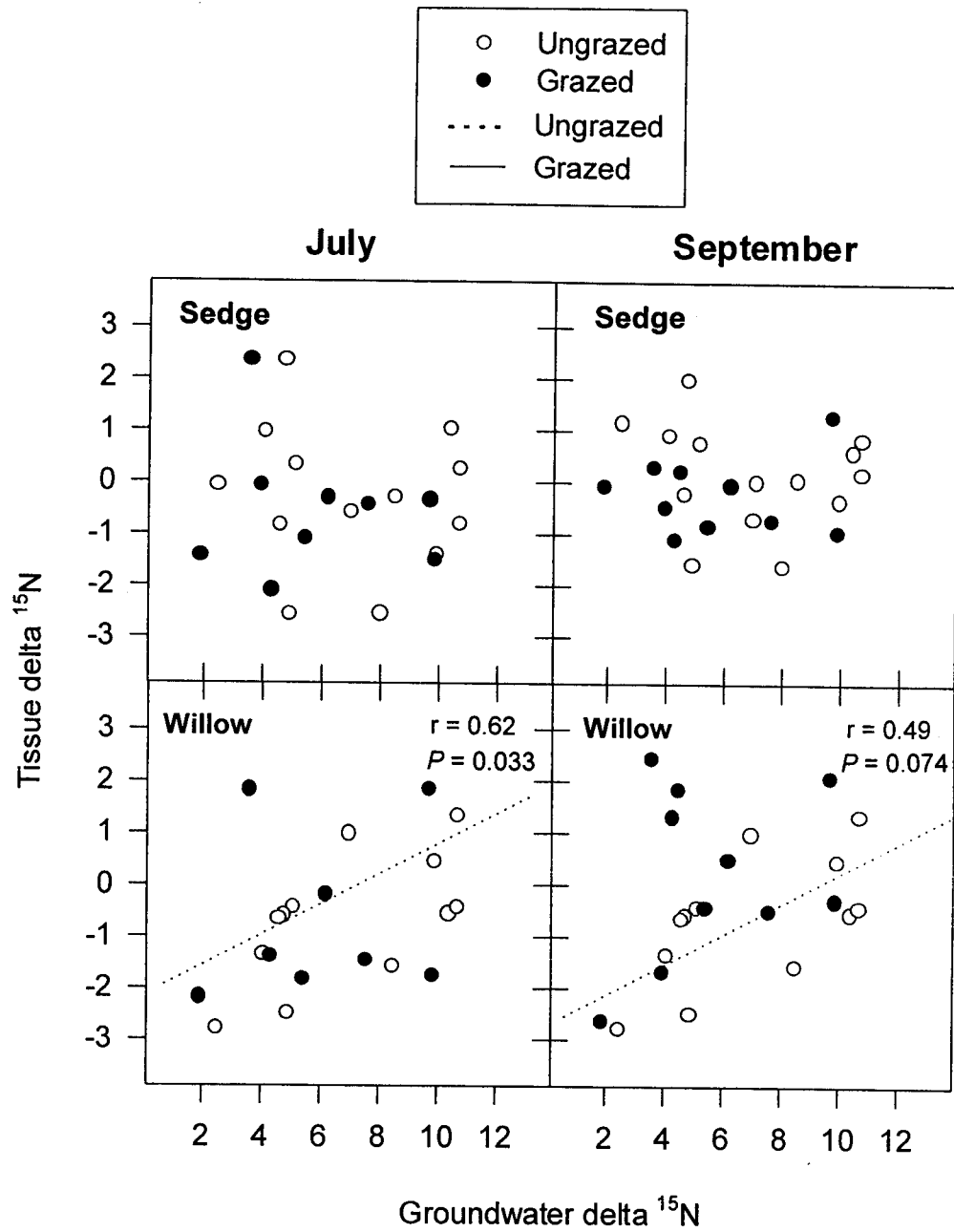


Fig. 4. Correlations between delta ¹⁵N values of groundwater and sedge and willow tissue in July and September of 1998 in grazed and ungrazed plots of Moraine and Horseshoe Parks.

in some cases could range between -10 and +10% (Virginia and Delwiche 1982; Vitousek et al. 1989; Nadelhoffer and Fry 1994; Frank and Evans 1997). In general, the differences between plant tissue $\delta^{15}\text{N}$ and groundwater $\delta^{15}\text{N}$ in our study were consistently higher (c. 7%) than the differences between plant tissue $\delta^{15}\text{N}$ and total soil $\delta^{15}\text{N}$ (c. 2%), which suggests that both willow and sedge plants in our sites may rely mostly on uptake of soil N as opposed to groundwater N.

Effect of Landscape Position on N Sources

During 1997, we found that sedge tissue in lower landscape positions consistently exhibited enriched $\delta^{15}\text{N}$ values when compared to sedge plants growing in upland positions in the landscape (Table 1). In contrast, $\delta^{15}\text{N}$ values of willow tissue were not significantly different between landscape positions (Table 1). This evidence suggests that the shallow-rooted graminoid may be relying more on soil N and acquiring smaller proportions of river-groundwater N in upper landscape positions in comparison to the woody willow species. This general interpretation is supported by our additional finding during 1998, in which a significant correlation was observed for sedge tissue $\delta^{15}\text{N}$ and water table depth (Fig. 1), indicating that the lower the water table (i.e., less access to groundwater N), the more depleted the $\delta^{15}\text{N}$ values of sedge tissue. To the contrary, willow leaf $\delta^{15}\text{N}$ values were not associated with water table depth (Fig. 1) with the exception of grazed plants in July. These findings suggest that the deeper rooting characteristics of willow may allow these plants to tap groundwater N independent of landscape position, but grazing may limit the access to groundwater in upper landscape sites. Similar to our results, previous studies have reported higher tissue $\delta^{15}\text{N}$ values in plants growing in lower landscape positions in comparison to plants in upper landscape positions (Garten 1993; Sutherland et al. 1993).

The observations presented here that landscape position influences facets of mineral nutrition of riparian vegetation are corroborated by a previous study conducted by Alstad et al. (1999) addressing water sources of these same species in the same experiment. Measurements of $\delta^{18}\text{O}$ showed that sedge plants in upper landscape positions relied strongly on rainwater acquired from upper soil layers, while sedge plants located adjacent to streams and willow plants in both landscape positions, primarily use streamwater. The agreement between the patterns of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in plant tissue strongly

supports our hypotheses regarding the patterns of N uptake by willow and sedge in our study sites. These findings further demonstrate the usefulness of stable isotopes in ecological studies, since we were not able to detect any influence of landscape position or herbivory on plant N sources based solely on the non-isotopic data we collected.

Effect of Herbivory on N Sources

Our findings also suggest that elk herbivory may have a significant effect on the patterns of N uptake by willow plants. As indicated by the correlations between groundwater $\delta^{15}\text{N}$ and willow tissue $\delta^{15}\text{N}$ (Fig. 4), grazed willow plants may have a more limited access to groundwater N than willow plants protected from herbivory. These findings were corroborated by a parallel investigation, based on non-isotopic techniques, of the N balance of willow plants conducted in our study site, which indicated that grazed willow plants may not have as much access to groundwater N when compared to ungrazed willow plants. The findings from this parallel study demonstrated that ungrazed willow plants could take up at least 7% of the annual requirements of N from the groundwater, which was higher ($P < 0.001$) than that of grazed plants, which averaged only 4% (Peinetti et al. 2001).

We suggest that grazed willow plants may have less access to groundwater N due to a less developed rooting system. A carbon-balance study conducted in our site indicated that grazed willow plants might allocate less carbon to the rooting system, in comparison to willow plants protected from herbivory (R. Peinetti, Natural Ecology Resources Laboratory, personal communication). In addition, several previous studies have reported that heavily grazed plants often allocate a smaller proportion of their photosynthetically fixed carbon to belowground structures than do ungrazed or lightly grazed plants (Detling 1987; Briske and Richards 1995; Detling 1998).

In nitrogen-limited systems, competition for soil N by coexisting plants may cause plants to resort to different sources of N in order to meet their physiological requirements (Welker et al. 1991; Schulze et al. 1994; Michelsen et al. 1996). Menezes et al. (2001) conducted fertilization experiments in our study sites and found that N availability significantly limits growth and uptake of N by willow plants. Therefore, the ability of plants to use more than one source may be of significant importance for plant growth, development, and survival.

A reduced ability by grazed willow plants to access different sources of N could significantly reduce the ability of willow plants to respond to herbivory.

Summary and Conclusions

We summarize our findings in Fig. 5, which illustrates the suggested patterns of N uptake by grazed and ungrazed willow and sedge plants in different landscape positions, and shows the average $\delta^{15}\text{N}$ values of N sources and plant tissue under those treatments during the growing season of 1998. In conclusion, we suggest that shifts in landscape hydrology, due to reductions in beaver activity and a warming and drying trend, combined with intense elk herbivory, may have a compounding effect on willow by increasing the frequency of drought stress and possibly reducing the availability of nitrogen. Since willow growth and uptake of N is limited by N availability in our sites, reductions in stream water N sources could increase the limitation of N to these plants and lead to lower rates of photosynthesis and growth. We suggest that these effects explain in part the sensitivity of willow communities to elk grazing and their decline across elk winter ranges in Rocky Mountain National Park.

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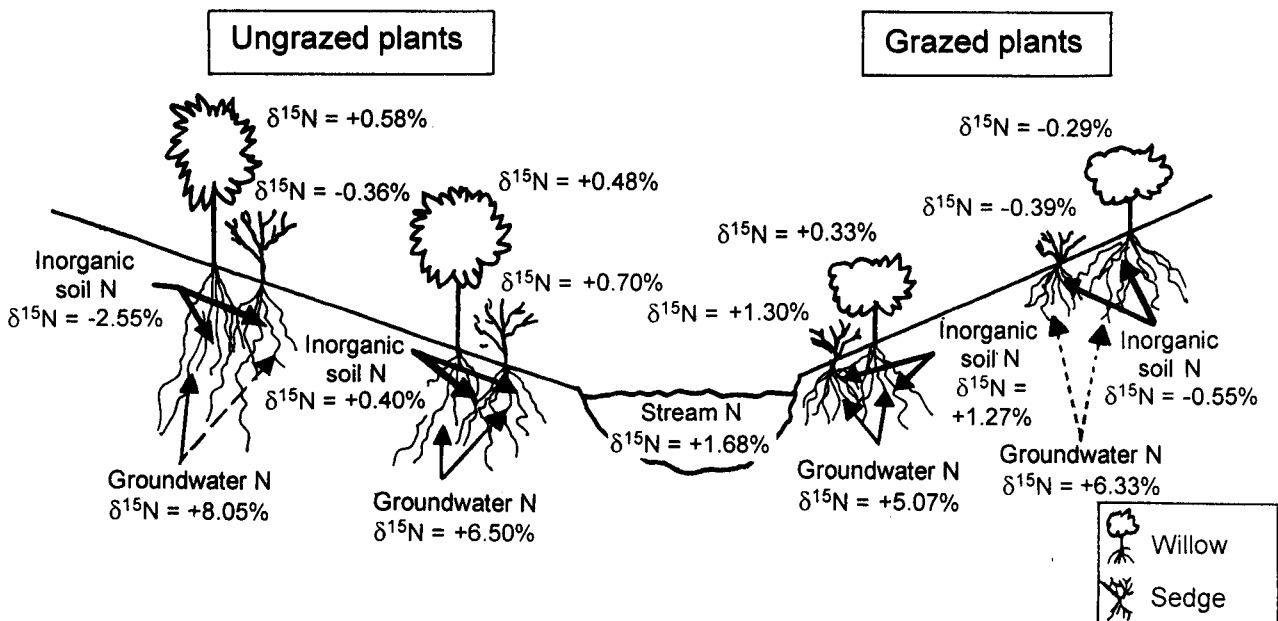


Fig. 5. Hypothetical patterns of N uptake by grazed and ungrazed willow and sedge plants in upper and lower landscape positions of Moraine and Horseshoe Parks.

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