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REGULAR ARTICLE

# Plant physiological responses to hydrologically mediated changes in nitrogen supply on a boreal forest floodplain: a mechanism explaining the discrepancy in nitrogen demand and supply

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**Abstract** A discrepancy between plant demand and soil supply of nitrogen (N) has been observed in early successional stages of riparian vegetation in interior Alaska. We hypothesized that a hydrologically mediated N supply serves as a mechanism to balance this apparent deficiency of plant N supply. To test this hypothesis, we conducted a tracer experiment and measured the activity of nitrate reductase (NRA) over the summer on the early successional floodplain of the Tanana River in interior Alaska. Isotopic data showed that river-/groundwater was an important source of plant water and that hyporheic N could be absorbed by early successional species. Plant NRA generally increased as the growing season progressed, and $NO_3^-$-N availability increased. Both *Salix interior* Rowlee and *Populus balsamifera* L. used $NO_3^-$-N, and the timing of plant NRA relative to river discharge chemistry and soil $NO_3^-$-N concentrations, strongly suggest that plant uptake of $NO_3^-$-N is coupled to fluvial dynamics. Moreover, this physiological function helps explain the apparent discrepancy between N mineralization and productivity in these riparian ecosystems, and demonstrates that plant N availability in these riparian stands is under significant hydrological control.

**Keywords** Floodplain · Hyporheic nitrogen (N) · N uptake · Plant nitrate ($NO_3^-$-N) use · River discharge chemistry · Seasonal change

## Introduction

Riparian ecosystems along the Tanana River represent the most productive forests in interior Alaska (Van Cleve et al. 1993). Soil inorganic nitrogen (N) is typically dominated by ammonium ($NH_4^+$-N), with very low concentrations of nitrate ($NO_3^-$-N) and negligible rates of nitrification in these forests (Klingensmith and Van Cleve 1993; Kielland et al. 2006a). Moreover, because of the arid climate, atmospheric N inputs via wet/dry deposition are very low (0.065±0.018 $g\,m^{-2}\,year^{-1}$; National Atmospheric Deposition Program 2007). Consequently, internal recycling of N (N mineralization) has been considered a major process for N supply to plants in these forests (Kielland et al. 2006a; Valentine et al. 2006). However, budget estimates of the relative magnitude of the N supply and vegetation N requirement

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suggest that the commonly recognized mechanisms of N supply (N mineralization, N-fixation, and N deposition) account for less than half of the N requirement of riparian vegetation (Ruess et al. 1996; Lisuzzo et al. 2008). This discrepancy between soil N supply and plant N demand suggests that additional mechanisms of N supply are operating (Kielland 2001). One of these mechanisms is the direct absorption of organic N in the form of amino acids (McFarland et al. 2002; Kielland et al. 2006b), although this process is far more important in late successional forests in which the concentrations and production of soil amino acids is high (Kielland et al. 2006b, 2007). Riparian communities along the Tanana River are strongly influenced by the river, as indicated by the tight coupling of river stage and groundwater depth (Clilverd et al. 2008), as well as the $\delta^{18}O$ signatures of riparian species (such as *Salix* sp. and *Populus* sp.). Recent estimates of N flux via hyporheic water flow suggest that this mechanism could double the N supply to riparian plant communities, effectively balancing the vegetation N budget (Clilverd et al. 2008; Lisuzzo et al. 2008). However, no study of these ecosystems has tried to explicitly link plant physiological responses to N availability with seasonal changes in hydrology that may control this N supply.

In this study, we examined whether N supplied via hyporheic flow could serve as an additional mechanism of plant N uptake for early successional species in boreal floodplain forests. We hypothesized that vegetation responses would be timed to the hydrological trajectory of an increasing $NO_3^-$-N supply during mid-season, rather than to the temporal pattern of nitrification, which reaches maximum rates in early June (Fig. 1). To test this hypothesis, we conducted a $^{15}N$ tracer experiment to demonstrate the linkage between N flux in hyporheic water and plant N uptake, and then investigated seasonal patterns of plant $NO_3^-$-N assimilation and soil $NO_3^-$-N pool size under natural N conditions.

We used river discharge dynamics and previously published information on river water chemistry and hyporheic N flux (Clilverd et al. 2008; Lisuzzo et al. 2008) to couple these hydrological data with plant physiological responses. We focused on *in vivo* nitrate reductase activity (NRA) as an indicator of $NO_3^-$-N assimilation (Koyama and Tokuchi 2003) because, in contrast to successional soils, $NO_3^-$-N concentrations in river and groundwater are several fold higher than that of $NH_4^+$-N (Clilverd et al. 2008). Nitrate reductase (NR) is a substrate-inducible enzyme, and the capacity to induce NR varies markedly among plant species (e.g., Gebauer et al. 1988). Thus, we focused our measurements on two dominant riparian species: sandbar willow (*Salix interior* Rowlee), which is typically found on the youngest terraces adjacent to the river, and balsam poplar (*Populus balsamifera* L.), which dominates on older terraces.

## Materials and methods

### Study site

This study was carried out on the Tanana River floodplain in the Bonanza Creek Long Term Ecological Research sites, approximately 20 km southwest of Fairbanks, Alaska, USA (Fig. 2; 64°40′33″N, 148°17′19″W). The climate is strongly continental and the area lies within a rain shadow created by the Alaska Range approximately 100 km to the south. Temperature extremes range from −50°C in winter to >+30°C during the summer, with an average of −3.3°C. Average annual precipitation is 269 mm, 37% of which falls as snow. Snow covers the ground for 6 to

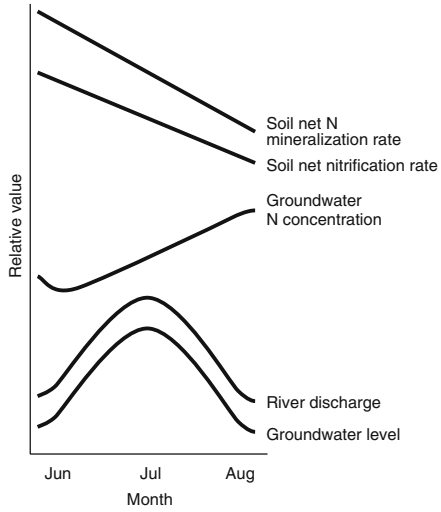


**Fig. 1** Schematic diagram of seasonal changes in environmental factors that can influence soil $NO_3^-$-N availability in the floodplain of the Tanana River, interior Alaska (Kielland et al. 2006a; Clilverd et al. 2008)

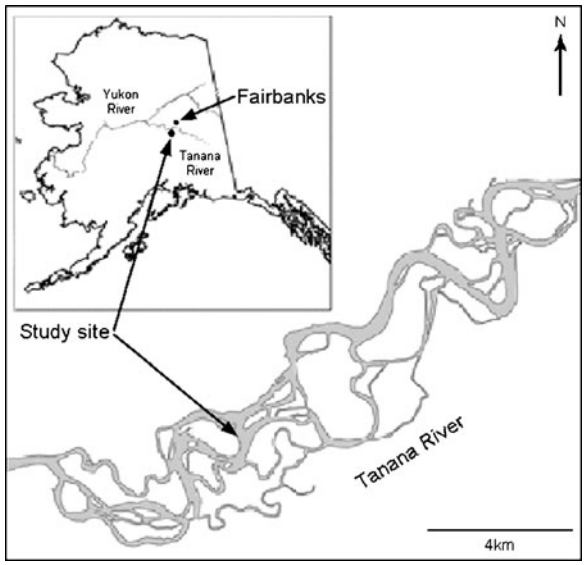


**Fig. 2** Location of the study site. The plant and soil samplings were conducted on the floodplain of the Tanana River, approximately 20 km southwest of Fairbanks, Alaska, USA

7 months of the year, from mid-October until early or mid-April. During the study period, the temperature ranged from 1.8°C to 29.5°C, with average of 16.1°C, and total precipitation was 142.7 mm (Fig. 3a, b; Bonanza Creek LTER Database, http://www.lter.uaf.edu/data_detail.cfm?datafile_pkey=1). River discharge data during the study period were obtained from the USGS Real-Time Water Data for station 15485500 at Fairbanks, Alaska (Fig. 3c; 64°47′34″N, 147°50′20″W; http://waterdata.usgs.gov/ak/nwis/uv/?site_no=15485500). The ground water depth data at the adjacent LTER plot were obtained from the Bonanza Creek LTER Database (http://www.lter.uaf.edu/data_detail.cfm?datafile_pkey=171)

The floodplain forest provides a typical example of primary succession (Chapin et al. 2006). Newly formed alluvial bars near the active channels are first colonized by willow (*Salix* spp.) and horsetail (*Equisetum* spp.). Thin-leaf alder (*Alnus tenuifolia* Nutt.) invades the willow/horsetail community, and alder/willow forest appears generally on a higher terrace than the willow/horsetail community. The alder/willow forest is followed by balsam poplar (*P. balsamifera*), white spruce [*Picea glauca* (Moench) Voss], and black spruce [*Picea mariana* (Mill.) Britton, Sterns & Poggenb.] in that order. The latter successional communities are found on the higher and farther terraces from the active channels.

The soils in early floodplain succession are sandy, with a thin silt loam layer on the surface. On older terraces (in later succession), the soils are predominantly silt-textured. The soils are classed as Typic Cryofluvents (Orthic Regosols; Viereck et al. 1993). In the oldest stages of succession dominated by coniferous forests (white and black spruce), the silt loam soils are cold and wet, and in the case of black spruce stands, often underlain by shallow permafrost (Van Cleve et al. 1993). Soils in these stages of succession are classed as

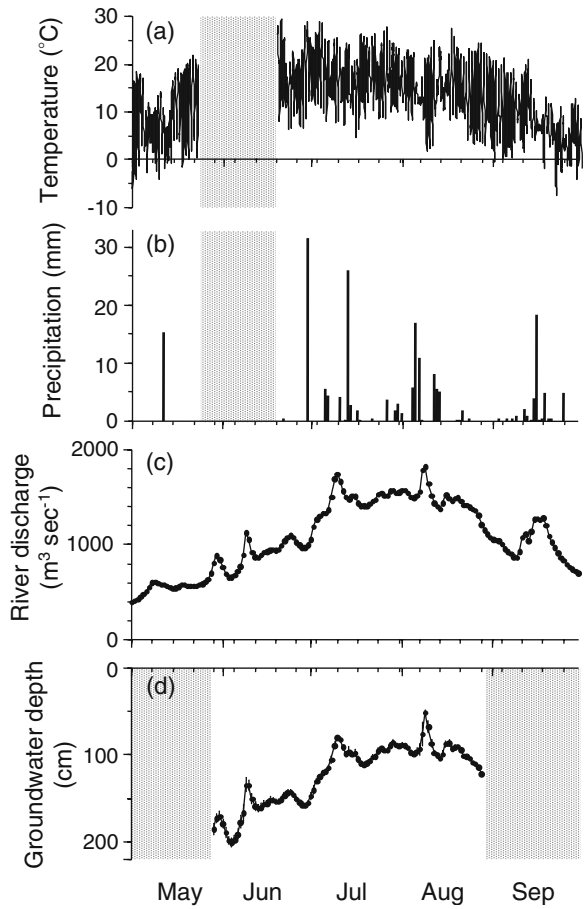


**Fig. 3** Changes in climate conditions, river discharge, and ground water depth during the growing season of the study year. **a** Hourly mean temperature and **b** daily precipitation data were from the nearest weather station of the BNZ-LTER site. Data from 24 May to 18 June 2007 (*shaded area*) were unavailable. **c** The river discharge data were obtained from the Web site of the USGS Real-Time Water Data for the period 1 May to 30 September 2007. **d** The ground depth data were obtained from the LTER plot adjacent to the study plot. Data before 28 May and after 29 August 2007 (*shaded area*) were unavailable

Histic Pergelic Cryaquepts (Gleysolic Static Cryosols; Viereck et al. 1993). Soil carbon (C) and N contents are very low at the initial stage and increase with succession, whereas soil pH decreases. Similarly, soil heat sums decrease across succession, reflecting the insulative effects of organic matter accumulation, a continuous moss cover, and an eventual dominance of permafrost (Chapin et al. 2006).

### Linkage between N flux in hyporheic water and plant N uptake

A $^{15}N$ injection experiment was conducted to investigate the influence of hyporheic N supply and plant N acquisition *in situ*. The purpose of this experiment was to demonstrate that inorganic N ($NH_4^+$-N and $NO_3^-$-N) originating in groundwater beneath the rooting horizon may be advected to plant roots and absorbed; we did not attempt to estimate the quantitative importance of hyporheic N flux to plants *per se*. A slotted (2 mm) 3-m-long PVC pipe (5 cm I.D.) was buried horizontally in the alluvium at 1.3 m depth (below the average rooting horizon of *S. interior*: 0.7 m) in May 2006, prior to river level rise. The pipe was covered with a 20-cm layer of quartz sand to minimize siltation and the trench was backfilled with natural alluvium. Each end of the pipe was fitted with an elbow that reached above ground. After the river level rose to flood the pipe, but before the groundwater level reached plant roots, we added 1 L $^{15}N$-labeled $NH_4NO_3$ (1 mM, 99% enrichment), which was circulated through the pipe using two hoses (1 cm I.D.) connected to a peristaltic pump. The N concentration of the label was high, as we estimated the addition would be diluted quickly up to 1000-fold. After 2 weeks, we sampled leaf tissue of *S. interior* on a 15 m (wide)×30 m (long) grid downstream of the pipe. Samples were collected systematically at every 1 m and were pooled within each distance strata from the pipe. Control samples were obtained upstream of the pipe. Samples were analyzed on a Europa 20–20 mass spectrometer at the University of Alaska, Fairbanks, Alaska, USA.

### Seasonal changes in soil $NO_3^-$-N pool size and plant NRA

Seasonal changes in soil $NO_3^-$-N availability and plant NRA were investigated in early and mid-successional stands. The early successional stand was dominated by *S. interior*, which mainly grew on the lowest terrace, near the active river channel. The mid-successional stand was dominated by *P. balsamifera* with an understory of alder (*A. tenuifolia*). The mid-successional stands are typically found on terraces that are about 0.5–1.0 m higher than the terrace adjacent to the river, i.e., the early successional stands. Plant and soil samples were collected six times during the growing season (from June to August) of 2007. At each sampling date, the leaves and fine roots (D<2 mm) of *S. interior* and *P. balsamifera* were collected ($n$=5). Leaf samples of *S. interior*, a shrub or small tree species, were randomly collected from the whole canopy. Leaves of *P. balsamifera* were sampled from fully lit condition of a consistent height (1–2 m) and aspect; the sample collection was carried out on the edge of the upper terrace from the aspect that faced to the shrub community on the lower terrace. All of the leaf samples were collected from the primary flush. Surface soil samples (0–10 cm depth from the surface of the mineral layer) were collected simultaneously with plant samples from the areas within a 50 cm radius of each sample tree. An individual, once chosen, was not repeatedly sampled to avoid sampling effects. Samples were collected from 10:00 to 14:00 to avoid the effect of diurnal changes in leaf NRA and were kept on ice until laboratory analysis.

We measured *in vivo* NRA under both saturating and ecological (limiting) conditions, using modified versions of the Jaworski method (Jaworski 1971; Thomas and Hilker 2000; Koyama and Tokuchi 2003). NRA(+$NO_3$) was measured as the rate of nitrite ($NO_2^-$-N) production in incubation buffer containing non-limiting $NO_3^-$-N. NRA(-$NO_3$) was determined in parallel measurements with incubation buffer without $NO_3^-$-N added to examine the relative magnitude of *in situ* $NO_3^-$-N assimilation.

Root samples were washed with tap water followed by deionized water to remove soil. About 100 mg (fresh weight) of the leaf laminae and fine roots were cut into small fragments (2.5-mm-diameter disks or about 4-mm$^2$ segments of leaves, and about 2-mm-long roots) and transferred to test tubes. Incubation buffer (5 ml) was added, and the tube contents were vacuum infiltrated. The composition of the incubation buffer was 0.1 mol $L^{-1}$ $KNO_3$ [for NRA(+$NO_3$) only], 0.1 mol $L^{-1}$ $KH_2PO_4$, and 1.5%

1-propanol; the pH was adjusted to about 7.5 using a NaOH solution. The samples were incubated for 1 h at 30°C in the dark. Enzyme activity was halted by placing the sample vials in hot water (>80°C). The concentration of $NO_2^-$-N in the incubation buffer was measured colorimetrically using diazotization (Keeney and Nelson 1982). The effect of plant pigments was compensated for by measuring controls lacking N-naphtylethylene diamine dihydrochloride (Gebauer et al. 1998). A fraction of each leaf sample was oven-dried at 105°C and then weighed to calculate the activity per unit dry weight.

The remaining leaves and roots were dried and ground. About 100 mg of ground sample was extracted with 10 ml deionized water for 1 h at 45°C. The extract was filtered and the concentration of $NO_3^-$-N in the extract analyzed in an AutoAnalyzerIII (BLTec, Osaka, Japan). Plant pigments in extracts may cause overestimation of $NO_3^-$-N concentration, and other unknown compounds in the extracts may inhibit reduction of $NO_3^-$-N to $NO_2^-$-N, which is colorimetrically measured in the AutoAnalyzerIII (data not shown). A standard addition method was applied to compensate for the effects of pigments and other compounds in the extract as necessary when the sample composition was unknown or complex and might affect the analytical signal (Harris 2007). In this method, standard solutions of known concentrations were added to each extract, and from the increases in signal (i.e., absorbance), concentration in the original extract was calculated. The concentration of total N in the ground sample was analyzed using a N/C analyzer (NC-900; Sumika, Osaka, Japan).

For soil $NO_3^-$-N content measurement, a 5-g sample was extracted with 50 ml deionized water, then filtered. The $NO_3^-$-N in the extract was determined using a Technicon Autoanalyzer following Cd reduction ($NO_3^-$-N + $NO_2^-$-N) using the Gries-Ilosvay method (Mulvaney 1996). Soil $NO_3^-$-N concentrations were calculated as N mass per unit soil weight.

### Statistical analysis

Two-way ANOVA was conducted to detect species difference and seasonal changes in NRA(+$NO_3$), NRA (−$NO_3$), $NO_3^-$-N concentrations, and N concentrations in leaves and roots. Similarly, two-way ANOVA was carried out to compare the soil $NO_3^-$-N concentration and soil water content among stands and sampling dates. All statistical analyses were done using the statistical package R version 2.8 (available at http://www.R-project.org).

## Results

### Seasonal changes in river discharge

The seasonal changes in river discharge showed a gentle peak from late July to early August 2007 (Fig. 3c). Three rapid, though moderate, increases in river discharge in early June, early July, and early August were associated with precipitation events (Fig. 3). The ground water depth at the adjacent LTER plot changed in parallel with the river discharge (Fig. 3d).

### Linkage between hyporheic N flux and plant N uptake

Two weeks after the $^{15}$N-labeled $NH_4NO_3$ injection, we observed a sharp peak of $\delta^{15}$N enrichment in the leaves of willow at 4 m downstream of the isotope-injection pipe (Fig. 4). The enrichment dropped off quickly, and at 6 m downstream, no difference was seen in the $\delta^{15}$N signature between treatment and control plants. Samples collected after 1 month gave a similar result, with enrichment being extended approximately 10 m downstream of the pipe (data not shown).

### Seasonal changes in plant NRA and soil $NO_3^-$-N pool size

The activity of NR in the presence of added $NO_3^-$-N [i.e., NRA(+$NO_3$)] was significantly higher in *P. balsamifera* than in *S. interior*, both in the leaves and roots throughout the sampling period (Fig. 5a, b, Table 1). Seasonal changes in leaf NRA(+$NO_3$) were significant and similar in the two species since no interaction was found between species and sampling dates (Table 1). In contrast, root NRA(+$NO_3$) showed no significant seasonal change.

The natural activity of NR, that is, in the absence of added $NO_3^-$-N [NRA(−$NO_3$)] was also significantly higher in *P. balsamifera* than in *S. interior*, both in leaves and roots throughout the sampling period

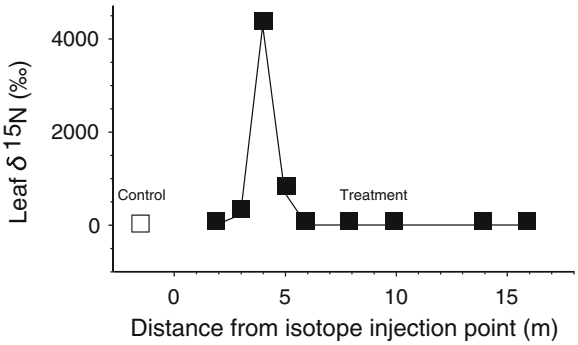


**Fig. 4** Relationship of $\delta^{15}N$ in *S. interior* leaves to distance from the point that $^{15}N$ tracer was injected into the groundwater. $^{15}N$-labeled $NH_4NO_3$ was added to the groundwater before the water table reached the rooting zone of *S. interior* in early spring, and leaves were collected 2 weeks after the isotope injection, when the water table rose to the plant rooting zone. Distance from the isotope injection point indicates upstream direction for control (*empty square*), and downstream direction for treatment (*filled square*; only data up to 16 m are shown) samples

(Fig. 5c, d, Table 1). In contrast to NRA(+$NO_3$), NRA (−$NO_3$) was higher in the roots than in the leaves of both species. NRA(−$NO_3$) in roots varied significantly among sampling days and generally increased as the growing season progressed.

In contrast to enzyme activities, plant $NO_3^-$-N concentrations in leaves and roots were significantly higher in *S. interior* than in *P. balsamifera* (Fig. 5e, f, Table 1). Leaves showed significant seasonal changes, while roots did not (Table 1).

Plant N concentrations in *P. balsamifera* were significantly higher than in *S. interior*, particularly in roots, until the end of the growing season (Fig. 5g, h, Table 1). Leaf N concentration was higher than root N concentration in both species. Root N concentration decreased significantly at the end of the season in *P. balsamifera*, while roots of *S. interior* retained a nearly constant N concentration throughout.

$NO_3^-$-N concentrations were significantly higher in the soils associated with *P. balsamifera* than those with *S. interior* (Fig. 6a, Table 1). Soil $NO_3^-$-N content showed significant seasonal patterns, and these patterns were similar between species. The seasonal patterns in soil water content differed between stands (Fig. 6b, Table 1). The seasonal pattern of water content in soils associated with *S. interior* showed a single peak (from late July to early August) during the sampling period. In contrast, soil moisture in the *P. balsamifera* stands peaked in the first half of July, after which water content decreased until early August, when soil moisture increased again due to precipitation.

## Discussion

### Linkage between N flux in hyporheic water and plant N uptake

The isotope experiment demonstrated that N dissolved in hyporheic water could be accessed by the riparian species *S. interior* (Fig. 4). This observation shows that advective movement of N is a source of plant N, and that variation in river discharge dynamics exhibits control over N supply and uptake by plants (cf. Fig. 1). Based on xylem sap $\delta^{18}O$ signatures, both *S. interior* and *P. balsamifera* ($\delta^{18}O = -16‰ \pm 2‰$) appear to derive approximately equal amounts of water from the river (−20‰) and summer precipitation (−10‰; Kielland unpublished data). Consequently, both species should have access to groundwater. However, we surmise that the direct contribution of hyporheic N flux is more important for species such as *S. interior* that grow on the newly formed silt bar adjacent to the river than for *P. balsamifera*, which is predominant on older, higher terraces. The main reason for this is that both $NO_3^-$-N concentration in hyporheic water and water table height decline with distance from the river (Clilverd et al. 2008), and the higher terrace, where *P. balsamifera* is predominant, supports higher rates of *in situ* N mineralization due to higher soil C content than that of the lower terrace (Kielland et al. 2006a).

### Species difference in $NO_3^-$-N use

The capacity to induce NR varies markedly among species, and there are species that have no capacity to use $NO_3^-$-N as a N source (Gebauer et al. 1988; Koyama and Tokuchi 2003; Smirnoff et al. 1984). Some previous works have demonstrated that a *Populus* species (*Populus tremuloides*) have capacity to use $NO_3^-$-N (Min et al. 1998, 1999; Rothstein et al. 2000). Our data from both *P. balsamifera* and *S. interior* regarding NRA and tissue $NO_3^-$-N concentrations suggest that both species have the capacity to take up and assimilate $NO_3^-$-N as a N source (Fig. 5a–f).

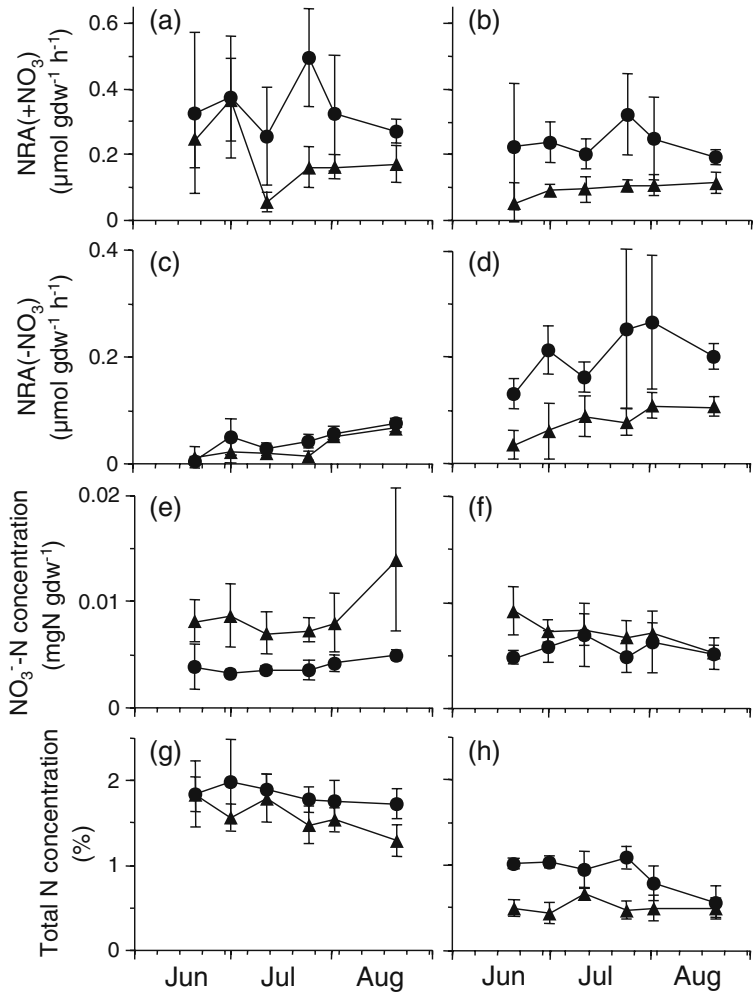


**Fig. 5** Seasonal changes in NRA(+$NO_3$), NRA(−$NO_3$), $NO_3^-$-N concentration, and N concentration in leaves and roots of *S. interior* and *P. balsamifera*. Means ± SD ($n$=5) are shown for **a** leaf NRA(+$NO_3$), **b** root NRA(+$NO_3$), **c** leaf NRA(−$NO_3$), **d** root NRA(−$NO_3$), **e** leaf $NO_3^-$-N concentration, **f** root $NO_3^-$-N concentration, **g** leaf N concentration, and **h** root N concentration. Circles and triangles indicate *P. balsamifera* and *S. interior*, respectively

The site of $NO_3^-$-N reduction in plants varies depending on species, developmental stage, and environment (Miller and Cramer 2004). We cannot specify what factor regulated the site of $NO_3^-$-N reduction in the two study species, but both species showed a similar pattern in their allocation of NRA. In both species, roots exhibited greater NRA than leaves throughout the study period in the absence of experimentally added $NO_3^-$-N for incubation (Fig. 5a–d) suggesting that $NO_3^-$-N was substantially assimilated in roots.

Both the leaves and roots of *P. balsamifera* had significantly higher NRA(+$NO_3$) and NRA(−$NO_3$) than did *S. interior* throughout the sampling period (Fig. 5a–d, Table 1). We surmise that this physiological trait is in response to the high rates of nitrification in stands with a substantial understory of alder (*A. tenuifolia*; Kielland et al. 2006a). Although the physiological capacity of *P. balsamifera* to absorb $NH_4^+$-N is greater than for $NO_3^-$-N, as is the case for many taiga tree species, *P. balsamifera* has a greater capacity for $NO_3^-$-N uptake than allopatric floodplain species such as alder (Chapin et al. 1986). In contrast, tissue $NO_3^-$-N concentrations were significantly higher both in leaves and roots throughout the sampling period in *S. interior* than in *P. balsamifera* (Fig. 5e–f, Table 1), suggesting that *S. interior* did not effectively assimilate $NO_3^-$-N in step with the absorption of $NO_3^-$-N (see also Fig. 4).

**Table 1** Results of the two-way ANOVA. For plants, NRA(+$NO_3$), NRA (−$NO_3$), $NO_3^-$-N concentration, and N concentration were compared between species and sampling dates

| | | | Leaf | | | Root | | | Soil | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | df | F | p | df | F | p | df | F | p |
| Plant | NRA(+$NO_3$) | species | 1 | 19.74 | <.01 | 1 | 43.60 | <.01 | | | |
| | | date | 5 | 3.51 | <.01 | 5 | 0.89 | 0.49 | | | |
| | | species*date | 5 | 1.90 | 0.00 | 5 | 0.85 | 0.52 | | | |
| | NRA(−$NO_3$) | species | 1 | 9.68 | <.01 | 1 | 60.26 | <.01 | | | |
| | | date | 5 | 22.95 | <.01 | 5 | 3.41 | 0.01 | | | |
| | | species*date | 5 | 2.29 | 0.06 | 5 | 1.13 | 0.36 | | | |
| | $NO_3^-$-N concentration | species | 1 | 55.34 | <.01 | 1 | 10.64 | <.01 | | | |
| | | date | 5 | 3.80 | <.01 | 5 | 1.89 | 0.11 | | | |
| | | species*date | 5 | 1.73 | 0.15 | 5 | 2.02 | 0.09 | | | |
| | N concentration | species | 1 | 14.36 | <.01 | 1 | 122.64 | <.01 | | | |
| | | date | 5 | 2.77 | 0.03 | 5 | 5.96 | <.01 | | | |
| | | species*date | 5 | 1.06 | 0.39 | 5 | 6.39 | <.01 | | | |
| Soil | $NO_3^-$-N content | species | | | | | | | 1 | 9.50 | <.01 |
| | | date | | | | | | | 5 | 5.84 | <.01 |
| | | species*date | | | | | | | 5 | 1.71 | 0.15 |
| | Water content | species | | | | | | | 1 | 10.68 | <.01 |
| | | date | | | | | | | 5 | 5.55 | <.01 |
| | | species*date | | | | | | | 5 | 2.49 | 0.04 |

For soil samples, $NO_3^-$-N content and water content were compared between species and among sampling dates

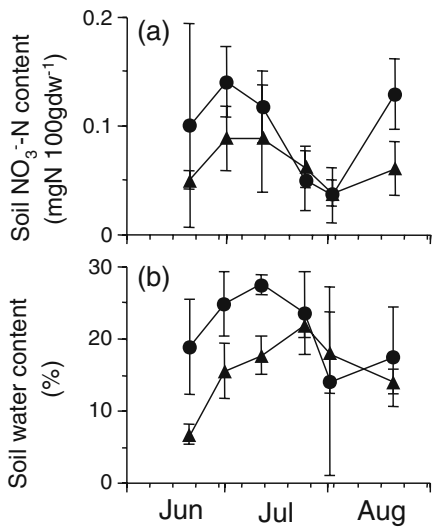


**Fig. 6** Seasonal changes in soil condition. Means ± SD ($n$=5) are shown for **a** $NO_3^-$-N content and **b** water content in soil associated with sample trees. Circles and triangles indicate *P. balsamifera* and *S. interior*, respectively

## Seasonal changes in plant $NO_3^-$-N use and soil $NO_3^-$-N availability

NRA(−$NO_3$) generally increased throughout the study period, both in leaves and roots of these two species, whereas leaf NRA(+$NO_3$) declined in mid-July and increased again, and root NRA(+$NO_3$) showed no significant change during the study period (Fig. 5a–d). In some temperate tree species, a sharp peak in leaf NRA(+$NO_3$) has been observed in the mid-leaf expansion period that is ascribed to compensation for the decline in leaf N concentration during leaf expansion (Koyama et al. 2008). Many other species also show maximum NRA(+$NO_3$) in the relatively early stages of leaf development (Gebauer et al. 1987; Högberg et al. 1986, 1992; Ohlson and Högbom 1993; Pearson and Ji 1994; Stadler and Gebauer 1992; Troelstra et al. 1995). In this study, no clear peak was observed in the early study period, possibly because leaf expansion occurs very rapidly in the boreal forest and had finished by the time our sampling started.

External environmental factors also influence seasonal changes in plant NRA. For example, high shoot NRA in *Deschampsia flexuosa* was observed in

early spring, and was partly attributable to low ambient temperature (Troelstra et al. 1995). In this study, the variability in temperature within a day (15.0±4.5°C) was larger than seasonal changes in daily mean temperature (from a minimum 10.3°C to a maximum 21.4°C). Therefore, the diurnal change in NRA related to light period is likely to be greater than the longer-term temporal change in NRA by temperature in this study site. Water availability is another factor that influences plant $NO_3^-$-N use, and the NRA of *Atriplex canescens* growing in an arid environment increased during the rainy season (Sisson and Throneberry 1986). However, water availability is unlikely to limit plant NRA at the study site as seasonal patterns of soil water contents were not mirrored by NRA in either *S. interior* or *P. balsamifera* (Figs. 5a–d and 6b), although the climate is continental and semiarid. Soil $NO_3^-$-N availability is the most frequently cited external factor for plant NRA, since NR is substrate-inducible. For example, temporal correspondence between NRA(+$NO_3$) and soil $NO_3^-$-N availability was observed in shoots of *D. flexuosa* (Troelstra et al. 1995) and needles of *Picea rubens* (Tjoelker et al. 1992). However, no clear correspondence was observed between the temporal changes in plant NRA and soil $NO_3^-$-N content in the two study species (Figs. 5a–d, 6a). NRA of *P. balsamifera* increased to some extent during the period in which soil $NO_3^-$-N content declined, which we suggest was a consequence of rapid $NO_3^-$-N uptake in this fast-growing species.

The seasonal patterns in soil water content differed statistically between two study species (Fig. 6b, Table 1). In the soils associated with *S. interior*, water content showed a single peak in late July, whereas water content in soils associated with *P. balsamifera* declined in the same period and fell to the lowest point at the beginning of August. The seasonal change of water content in soils associated with *S. interior* corresponded to river discharge and groundwater depth; they peaked from the end of July to the beginning of August (Figs. 3c, d and 6b). Therefore, water content of the surface soil in the lowest terrace where *S. interior* dominated was very likely to be influenced by changes in water table height, which are regulated by river discharge (Clilverd et al. 2008; see also Fig. 1). In contrast, the soils associated with *P. balsamifera* seemed less influenced by groundwater, since the distance from the surface soil to the water table increased with decline in water table height and terrace topography.

In contrast to water content, soil $NO_3^-$-N content had similar seasonal patterns in the two species (Fig. 6a, Table 1). Rhizosphere soils showed declines in $NO_3^-$-N concentrations in mid- to late season (from late July to early August), regardless of species (Fig. 1). Similar declines in $NO_3^-$-N concentration observed previously in groundwater suggest that the reduction is caused by a combination of plant $NO_3^-$-N uptake and high rates of denitrification (Clilverd et al. 2008).

## Contribution of hydrologically mediated N supply

The riparian soils along the Tanana River exhibit high hydraulic conductivity (Clilverd et al. 2008), resulting in much higher N flux to plant roots than would otherwise be indicated based on net rates of N mineralization (Kielland et al. 2006a; Lisuzzo et al. 2008). This may explain the sustainability of these highly productive communities despite the apparent inadequate N supply, as measured by net N mineralization (Kielland 2001; Ruess et al. 1996). Only 26% of the N requirement has been estimated to be supplied from N mineralization, N deposition, and N-fixation in the earliest successional stage, namely *S. interior* stands (Lisuzzo et al. 2008). Recently, several studies have shown that subsurface hydrology directly affects N availability in the floodplain forest of interior Alaska (Clilverd et al. 2008; Lisuzzo et al. 2008). The close relationships among river discharge, river N chemistry, and soil N chemistry suggest that hydrological processes exert significant control over plant N supply in these riparian systems. Moreover, the absolute flux of N forms such as $NO_3^-$-N is far greater than indicated by nitrification studies in the field, suggesting that $NO_3^-$-N may be far more important in the N economy of riparian species than hitherto considered. Our isotope injection experiment showed that *S. interior* had access to $NO_3^-$-N in the groundwater, and water table height and groundwater flow influenced the N supply to *S. interior* (Fig. 4). Our evidence also indicates that in early successional stages *S. interior* uses $NO_3^-$-N as an effective N source (Figs. 4 and 5). Although both soil nitrification rates and soil $NO_3^-$-N concentrations were very low in a *Salix* stand (Kielland et al. 2006a, 2007), our results indicate that hydrologically mediated $NO_3^-$-N

flow is an important mechanism for N supply in these ecosystems.

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