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4 Title:

5 Plant physiological responses to hydrologically mediated changes in nitrogen supply on

6 a boreal forest floodplain: a mechanism explaining the discrepancy in nitrogen demand and

7 supply

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1 **Abstract**

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

16

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

19

1 **Introduction**

2

3 Riparian ecosystems along the Tanana River represent the most productive forests in interior

4 Alaska (Van Cleve et al. 1993). Soil inorganic nitrogen (N) is typically dominated by

5 ammonium ($\text{NH}_4^+\text{-N}$), with very low concentrations of nitrate ($\text{NO}_3^-\text{-N}$) and negligible rates of

6 nitrification in these forests (Klingensmith and Van Cleve 1993; Kielland et al. 2006a).

7 Moreover, because of the arid climate, atmospheric N inputs via wet/dry deposition are very

8 low ($0.065 \pm 0.018 \text{ g m}^{-2} \text{ year}^{-1}$; National Atmospheric Deposition Program 2007).

9 Consequently, internal recycling of N (N mineralization) has been considered a major process

10 for N supply to plants in these forests (Kielland et al. 2006a; Valentine et al. 2006). However,

11 budget estimates of the relative magnitude of the N supply and vegetation N requirement

12 suggest that the commonly recognized mechanisms of N supply (N mineralization, N-fixation,

13 and N deposition) account for less than half of the N requirement of riparian vegetation (Ruess

14 et al. 1996; Lisuzzo et al. 2008). This discrepancy between soil N supply and plant N demand

15 suggests that additional mechanisms of N supply are operating (Kielland 2001). One of these

16 mechanisms is the direct absorption of organic N in the form of amino acids (McFarland et al.

17 2002; Kielland et al. 2006b), although this process is far more important in late successional

18 forests in which the concentrations and production of soil amino acids is high (Kielland et al.

19 2006b, 2007). Riparian communities along the Tanana River are strongly influenced by the

20 river, as indicated by the tight coupling of river stage and groundwater depth (Clilverd et al.

21 2008), as well as the $\delta^{18}\text{O}$ signatures of riparian species (such as *Salix* sp. and *Populus* sp.).

22 Recent estimates of N flux via hyporheic water flow suggest that this mechanism could double

23 the N supply to riparian plant communities, effectively balancing the vegetation N budget

24 (Clilverd et al. 2008; Lisuzzo et al. 2008). However, no study of these ecosystems has tried to

25 explicitly link plant physiological responses to N availability with seasonal changes in

26 hydrology that may control this N supply.

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

9 We used river discharge dynamics and previously published information on river water
10 chemistry and hyporheic N flux (Clilverd et al. 2008; Lisuzzo et al. 2008) to couple these
11 hydrological data with plant physiological responses. We focused on *in vivo* nitrate reductase
12 activity (NRA) as an indicator of NO_3^- -N assimilation (Koyama and Tokutchi 2003) because, in
13 contrast to successional soils, NO_3^- -N concentrations in river and groundwater are several fold
14 higher than that of NH_4^+ -N (Clilverd et al. 2008). Nitrate reductase (NR) is a substrate-inducible
15 enzyme, and the capacity to induce NR varies markedly among plant species (e.g., Gebauer et al.
16 1988). Thus, we focused our measurements on two dominant riparian species: sandbar willow
17 (*Salix interior* Rowlee), which is typically found on the youngest terraces adjacent to the river,
18 and balsam poplar (*Populus balsamifera* L.), which dominates on older terraces.

19

20 **Materials and methods**

21

22 Study site

23

24 This study was carried out on the Tanana River floodplain in the Bonanza Creek Long Term
25 Ecological Research sites, approximately 20 km southwest of Fairbanks, Alaska, USA (Fig. 2;
26 $64^\circ40'33''\text{N}$, $148^\circ17'19''\text{W}$). The climate is strongly continental and the area lies within a rain
27 shadow created by the Alaska Range approximately 100 km to the south. Temperature

1 extremes range from -50°C in winter to $> +30^{\circ}\text{C}$ during the summer, with an average of –
2 3.3°C . Average annual precipitation is 269 mm, 37% of which falls as snow. Snow covers the
3 ground for 6 to 7 months of the year, from mid-October until early or mid-April. During the
4 study period, the temperature ranged from 1.8°C to 29.5°C , with average of 16.1°C , and total
5 precipitation was 142.7 mm (Fig. 3a, b; Bonanza Creek LTER Database,
6 http://www.lter.uaf.edu/data_detail.cfm?datafile_pkey=1). River discharge data during the
7 study period were obtained from the USGS Real-Time Water Data for station 15485500 at
8 Fairbanks, Alaska (Fig. 3c; $64^{\circ}47'34''\text{N}$, $147^{\circ}50'20''\text{W}$;
9 http://waterdata.usgs.gov/ak/nwis/uv/?site_no=15485500). The ground water depth data at the
10 adjacent LTER plot were obtained from the Bonanza Creek LTER Database
11 (http://www.lter.uaf.edu/data_detail.cfm?datafile_pkey=171)

12 The floodplain forest provides a typical example of primary succession (Chapin et al.
13 2006). Newly formed alluvial bars near the active channels are first colonized by willow (*Salix*
14 spp.) and horsetail (*Equisetum* spp.). Thin-leaf alder (*Alnus tenuifolia* Nutt.) invades the
15 willow/horsetail community, and alder/willow forest appears generally on a higher terrace than
16 the willow/horsetail community. The alder/willow forest is followed by balsam poplar (*P.*
17 *balsamifera*), white spruce [*Picea glauca* (Moench) Voss], and black spruce [*Picea mariana*
18 (Mill.) Britton, Sterns & Poggenb.] in that order. The latter successional communities are found
19 on the higher and farther terraces from the active channels.

20 The soils in early floodplain succession are sandy, with a thin silt loam layer on the
21 surface. On older terraces (in later succession), the soils are predominantly silt-textured. The
22 soils are classed as Typic Cryofluvents (Orthic Regosols; Viereck et al. 1993). In the oldest
23 stages of succession dominated by coniferous forests (white and black spruce), the silt loam
24 soils are cold and wet, and in the case of black spruce stands, often underlain by shallow
25 permafrost (Van Cleve et al. 1993). Soils in these stages of succession are classed as Histic
26 Pergelic Cryaquepts (Gleysolic Static Cryosols; Viereck et al. 1993). Soil carbon (C) and N
27 contents are very low at the initial stage and increase with succession, whereas soil pH

1 decreases. Similarly, soil heat sums decrease across succession, reflecting the insulative effects
2 of organic matter accumulation, a continuous moss cover, and an eventual dominance of
3 permafrost (Chapin et al. 2006).

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

6
7 A ^{15}N injection experiment was conducted to investigate the influence of hyporheic N supply
8 and plant N acquisition *in situ*. The purpose of this experiment was to demonstrate that
9 inorganic N (NH_4^+ -N and NO_3^- -N) originating in groundwater beneath the rooting horizon may
10 be advected to plant roots and absorbed; we did not attempt to estimate the quantitative
11 importance of hyporheic N flux to plants *per se*. A slotted (2 mm) 3-m-long PVC pipe (5 cm
12 I.D.) was buried horizontally in the alluvium at 1.3 m depth (below the average rooting horizon
13 of *S. interior*: 0.7 m) in May 2006, prior to river level rise. The pipe was covered with a 20-cm
14 layer of quartz sand to minimize siltation and the trench was backfilled with natural alluvium.
15 Each end of the pipe was fitted with an elbow that reached above ground. After the river level
16 rose to flood the pipe, but before the groundwater level reached plant roots, we added 1 L
17 ^{15}N -labeled NH_4NO_3 (1 mM, 99% enrichment), which was circulated through the pipe using
18 two hoses (1 cm I.D.) connected to a peristaltic pump. The N concentration of the label was
19 high, as we estimated the addition would be diluted quickly up to 1000-fold. After 2 weeks, we
20 sampled leaf tissue of *S. interior* on a 15 m (wide) \times 30 m (long) grid downstream of the pipe.
21 Samples were collected systematically at every 1 m and were pooled within each distance strata
22 from the pipe. Control samples were obtained upstream of the pipe. Samples were analyzed on a
23 Europa 20-20 mass spectrometer at the University of Alaska, Fairbanks, Alaska, USA.

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

26
27 Seasonal changes in soil NO_3^- -N availability and plant NRA were investigated in early and mid-

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

17 We measured *in vivo* NRA under both saturating and ecological (limiting) conditions,
18 using modified versions of the Jaworski method (Jaworski 1971; Thomas and Hilker 2000;
19 Koyama and Tokuchi 2003). NRA(+NO₃) was measured as the rate of nitrite (NO₂⁻-N)
20 production in incubation buffer containing non-limiting NO₃⁻-N. NRA(-NO₃) was determined in
21 parallel measurements with incubation buffer without NO₃⁻-N added to examine the relative
22 magnitude of *in situ* NO₃⁻-N assimilation.

23 Root samples were washed with tap water followed by deionized water to remove soil.
24 About 100 mg (fresh weight) of the leaf laminae and fine roots were cut into small fragments
25 (2.5-mm-diameter disks or about 4-mm² segments of leaves, and about 2-mm-long roots) and
26 transferred to test tubes. Incubation buffer (5 ml) was added, and the tube contents were vacuum
27 infiltrated. The composition of the incubation buffer was 0.1 mol L⁻¹ KNO₃ [for NRA(+NO₃)

1 only], 0.1 mol L⁻¹ KH₂PO₄, and 1.5% 1-propanol; the pH was adjusted to about 7.5 using a
2 NaOH solution. The samples were incubated for 1 h at 30°C in the dark. Enzyme activity was
3 halted by placing the sample vials in hot water (> 80°C). The concentration of NO₂⁻-N in the
4 incubation buffer was measured colorimetrically using diazotization (Keeney and Nelson 1982).
5 The effect of plant pigments was compensated for by measuring controls lacking
6 N-naphtylethylene diamine dihydrochloride (Gebauer et al. 1998). A fraction of each leaf
7 sample was oven-dried at 105°C and then weighed to calculate the activity per unit dry weight.

8 The remaining leaves and roots were dried and ground. About 100 mg of ground sample
9 was extracted with 10 ml deionized water for 1 h at 45°C. The extract was filtered and the
10 concentration of NO₃⁻-N in the extract analyzed in an AutoAnalyzerIII (BLTec, Osaka, Japan).
11 Plant pigments in extracts may cause overestimation of NO₃⁻-N concentration, and other
12 unknown compounds in the extracts may inhibit reduction of NO₃⁻-N to NO₂⁻-N, which is
13 colorimetrically measured in the AutoAnalyzerIII (data not shown). A standard addition method
14 was applied to compensate for the effects of pigments and other compounds in the extract as
15 necessary when the sample composition was unknown or complex and might affect the
16 analytical signal (Harris 2007). In this method, standard solutions of known concentrations were
17 added to each extract, and from the increases in signal (i.e., absorbance), concentration in the
18 original extract was calculated. The concentration of total N in the ground sample was analyzed
19 using a N/C analyzer (NC-900; Sumika, Osaka, Japan).

20 For soil NO₃⁻-N content measurement, a 5-g sample was extracted with 50 ml deionized
21 water, then filtered. The NO₃⁻-N in the extract was determined using a Technicon Autoanalyzer
22 following Cd reduction (NO₃⁻-N + NO₂⁻-N) using the Gries–Ilosvay method (Mulvaney 1996).
23 Soil NO₃⁻-N concentrations were calculated as N mass per unit soil weight.

24
25 Statistical analysis

26
27 Two-way ANOVA was conducted to detect species difference and seasonal changes in

1 NRA(+NO₃), NRA (-NO₃), NO₃⁻-N concentrations, and N concentrations in leaves and roots.
2 Similarly, two-way ANOVA was carried out to compare the soil NO₃⁻-N concentration and soil
3 water content among stands and sampling dates. All statistical analyses were done using the
4 statistical package R version 2.8 (available at <http://www.R-project.org>).

5 6 **Results**

7 8 Seasonal changes in river discharge

9
10 The seasonal changes in river discharge showed a gentle peak from late July to early August
11 2007 (Fig. 3c). Three rapid, though moderate, increases in river discharge in early June, early
12 July, and early August were associated with precipitation events (Fig. 3). The ground water
13 depth at the adjacent LTER plot changed in parallel with the river discharge (Fig. 3d).

14 15 Linkage between hyporheic N flux and plant N uptake

16
17 Two weeks after the ¹⁵N-labeled NH₄NO₃ injection, we observed a sharp peak of δ¹⁵N
18 enrichment in the leaves of willow at 4 m downstream of the isotope-injection pipe (Fig. 4). The
19 enrichment dropped off quickly, and at 6 m downstream, no difference was seen in the δ¹⁵N
20 signature between treatment and control plants. Samples collected after 1 month gave a similar
21 result, with enrichment being extended approximately 10 m downstream of the pipe (data not
22 shown).

23 24 Seasonal changes in plant NRA and soil NO₃⁻-N pool size

25
26 The activity of NR in the presence of added NO₃⁻-N [i.e., NRA(+NO₃)] was significantly higher
27 in *P. balsamifera* than in *S. interior*, both in the leaves and roots throughout the sampling period

1 (Fig. 5a, b, Table 1). Seasonal changes in leaf $\text{NRA}(+\text{NO}_3)$ were significant and similar in the
2 two species since no interaction was found between species and sampling dates (Table 1). In
3 contrast, root $\text{NRA}(+\text{NO}_3)$ showed no significant seasonal change.

4 The natural activity of NR, that is, in the absence of added NO_3^- -N [$\text{NRA}(-\text{NO}_3)$] was
5 also significantly higher in *P. balsamifera* than in *S. interior*, both in leaves and roots
6 throughout the sampling period (Fig. 5c, d, Table 1). In contrast to $\text{NRA}(+\text{NO}_3)$, $\text{NRA}(-\text{NO}_3)$
7 was higher in the roots than in the leaves of both species. $\text{NRA}(-\text{NO}_3)$ in roots varied
8 significantly among sampling days and generally increased as the growing season progressed.

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

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

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

26 **Discussion**

27

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

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

17
18 Species difference in NO_3^- -N use

19
20 The capacity to induce NR varies markedly among species, and there are species that have no
21 capacity to use NO_3^- -N as a N source (Gebauer et al. 1988; Koyama and Tokuchi 2003;
22 Smirnov et al. 1984). Some previous works have demonstrated that a *Populus* species (*Populus*
23 *tremuloides*) have capacity to use NO_3^- -N (Min et al. 1998, 1999; Rothstein et al. 2000). Our
24 data from both *P. balsamifera* and *S. interior* regarding NRA and tissue NO_3^- -N concentrations
25 suggest that both species have the capacity to take up and assimilate NO_3^- -N as a N source (Fig.
26 5a–f).

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

7 Both the leaves and roots of *P. balsamifera* had significantly higher $\text{NRA}(+\text{NO}_3)$ and
8 $\text{NRA}(-\text{NO}_3)$ than did *S. interior* throughout the sampling period (Fig. 5a–d, Table 1). We
9 surmise that this physiological trait is in response to the high rates of nitrification in stands with
10 a substantial understory of alder (*A. tenuifolia*; Kielland et al. 2006a). Although the
11 physiological capacity of *P. balsamifera* to absorb NH_4^+ -N is greater than for NO_3^- -N, as is the
12 case for many taiga tree species, *P. balsamifera* has a greater capacity for NO_3^- -N uptake than
13 allopatric floodplain species such as alder (Chapin et al. 1986). In contrast, tissue NO_3^- -N
14 concentrations were significantly higher both in leaves and roots throughout the sampling
15 period in *S. interior* than in *P. balsamifera* (Fig. 5e–f, Table 1), suggesting that *S. interior* did
16 not effectively assimilate NO_3^- -N in step with the absorption of NO_3^- -N (see also Fig. 4).

17
18 Seasonal changes in plant NO_3^- -N use and soil NO_3^- -N availability

19
20 $\text{NRA}(-\text{NO}_3)$ generally increased throughout the study period, both in leaves and roots of these
21 two species, whereas leaf $\text{NRA}(+\text{NO}_3)$ declined in mid-July and increased again, and root
22 $\text{NRA}(+\text{NO}_3)$ showed no significant change during the study period (Fig. 5a–d). In some
23 temperate tree species, a sharp peak in leaf $\text{NRA}(+\text{NO}_3)$ has been observed in the mid-leaf
24 expansion period that is ascribed to compensation for the decline in leaf N concentration during
25 leaf expansion (Koyama et al. 2008). Many other species also show maximum $\text{NRA}(+\text{NO}_3)$ in
26 the relatively early stages of leaf development (Gebauer et al. 1987; Högbom et al. 1986, 1992;
27 Ohlson and Högbom 1993; Pearson and Ji 1994; Stadler and Gebauer 1992; Troelstra et al.

1 1995). In this study, no clear peak was observed in the early study period, possibly because leaf
2 expansion occurs very rapidly in the boreal forest and had finished by the time our sampling
3 started.

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

23 The seasonal patterns in soil water content differed statistically between two study
24 species (Fig. 6b, Table 1). In the soils associated with *S. interior*, water content showed a single
25 peak in late July, whereas water content in soils associated with *P. balsamifera* declined in the
26 same period and fell to the lowest point at the beginning of August. The seasonal change of
27 water content in soils associated with *S. interior* corresponded to river discharge and

1 groundwater depth; they peaked from the end of July to the beginning of August (Figs. 3c, d
2 and 6b). Therefore, water content of the surface soil in the lowest terrace where *S. interior*
3 dominated was very likely to be influenced by changes in water table height, which are
4 regulated by river discharge (Clilverd et al. 2008; see also Fig. 1). In contrast, the soils
5 associated with *P. balsamifera* seemed less influenced by groundwater, since the distance from
6 the surface soil to the water table increased with decline in water table height and terrace
7 topography.

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

14 Contribution of hydrologically mediated N supply

15
16
17 The riparian soils along the Tanana River exhibit high hydraulic conductivity (Clilverd et al.
18 2008), resulting in much higher N flux to plant roots than would otherwise be indicated based
19 on net rates of N mineralization (Kielland et al. 2006a; Lisuzzo et al. 2008). This may explain
20 the sustainability of these highly productive communities despite the apparent inadequate N
21 supply, as measured by net N mineralization (Kielland 2001; Ruess et al. 1996). Only 26% of
22 the N requirement has been estimated to be supplied from N mineralization, N deposition, and
23 N-fixation in the earliest successional stage, namely *S. interior* stands (Lisuzzo et al. 2008).
24 Recently, several studies have shown that subsurface hydrology directly affects N availability in
25 the floodplain forest of interior Alaska (Clilverd et al. 2008; Lisuzzo et al. 2008). The close
26 relationships among river discharge, river N chemistry, and soil N chemistry suggest that
27 hydrological processes exert significant control over plant N supply in these riparian systems.

1 Moreover, the absolute flux of N forms such as NO_3^- -N is far greater than indicated by
2 nitrification studies in the field, suggesting that NO_3^- -N may be far more important in the N
3 economy of riparian species than hitherto considered. Our isotope injection experiment showed
4 that *S. interior* had access to NO_3^- -N in the groundwater, and water table height and
5 groundwater flow influenced the N supply to *S. interior* (Fig. 4). Our evidence also indicates
6 that in early successional stages *S. interior* uses NO_3^- -N as an effective N source (Figs. 4 and 5).
7 Although both soil nitrification rates and soil NO_3^- -N concentrations were very low in a *Salix*
8 stand (Kielland et al. 2006a, 2007), our results indicate that hydrologically mediated NO_3^- -N
9 flow is an important mechanism for N supply in these ecosystems.

10

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17

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- 18

1 **Figure Captions**

2

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

6

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

9

10 **Fig. 3** Changes in climate conditions, river discharge, and ground water depth during the
11 growing season of the study year. **a** Hourly mean temperature and **b** daily precipitation data
12 were from the nearest weather station of the BNZ-LTER site. Data from 24 May to 18 June
13 2007 (shaded area) were unavailable. **c** The river discharge data were obtained from the Web
14 site of the USGS Real-Time Water Data for the period 1 May to 30 September 2007. **d** The
15 ground depth data were obtained from the LTER plot adjacent to the study plot. Data before 28
16 May and after 29 August 2007 (shaded area) were unavailable.

17

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

24

25 **Fig. 5** Seasonal changes in $\text{NRA}(+\text{NO}_3)$, $\text{NRA}(-\text{NO}_3)$, NO_3^- -N concentration, and N
26 concentration in leaves and roots of *S. interior* and *P. balsamifera*. Means \pm SD ($n = 5$) are
27 shown for **a** leaf $\text{NRA}(+\text{NO}_3)$, **b** root $\text{NRA}(+\text{NO}_3)$, **c** leaf $\text{NRA}(-\text{NO}_3)$, **d** root $\text{NRA}(-\text{NO}_3)$, **e**

1 leaf NO_3^- -N concentration, **f** root NO_3^- -N concentration, **g** leaf N concentration, and **h** root N
2 concentration. Circles (●) and triangles (▲) indicate *P. balsamifera* and *S. interior*, respectively.
3
4 **Fig. 6** Seasonal changes in soil condition. Means \pm SD ($n = 5$) are shown for **a** NO_3^- -N content
5 and **b** water content in soil associated with sample trees. Circles (●) and triangles (▲) indicate
6 *P. balsamifera* and *S. interior*, respectively.

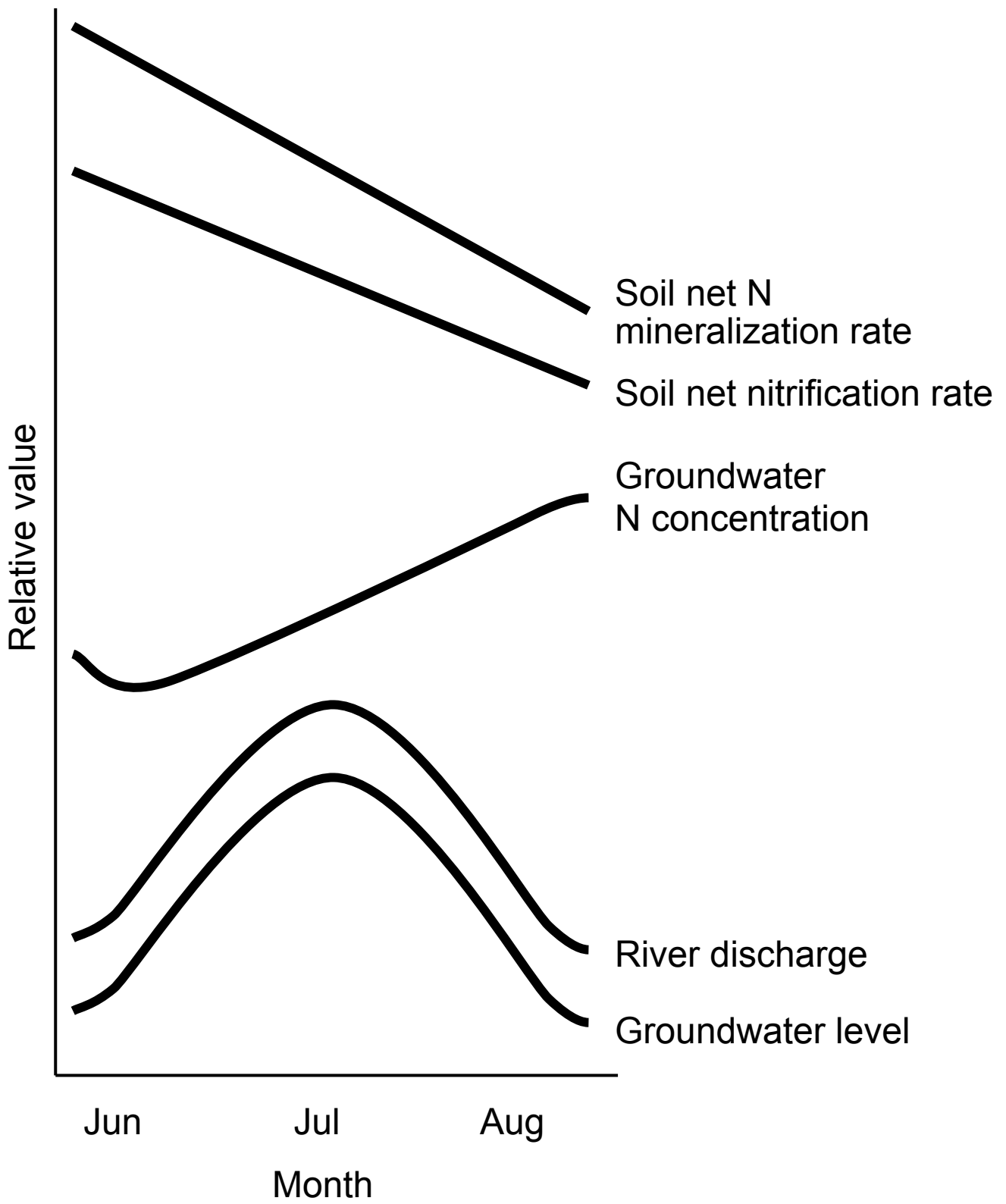


Fig. 1

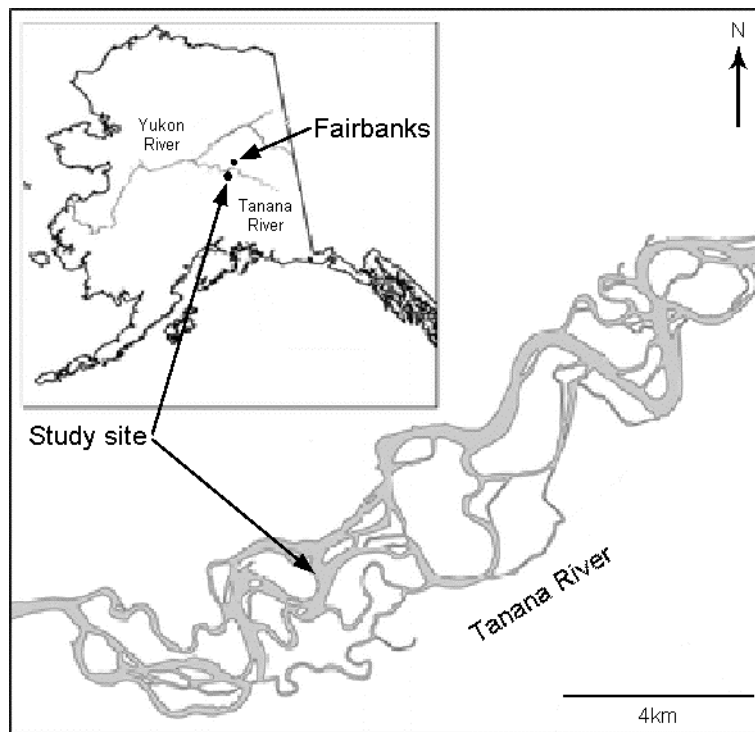


Fig. 2

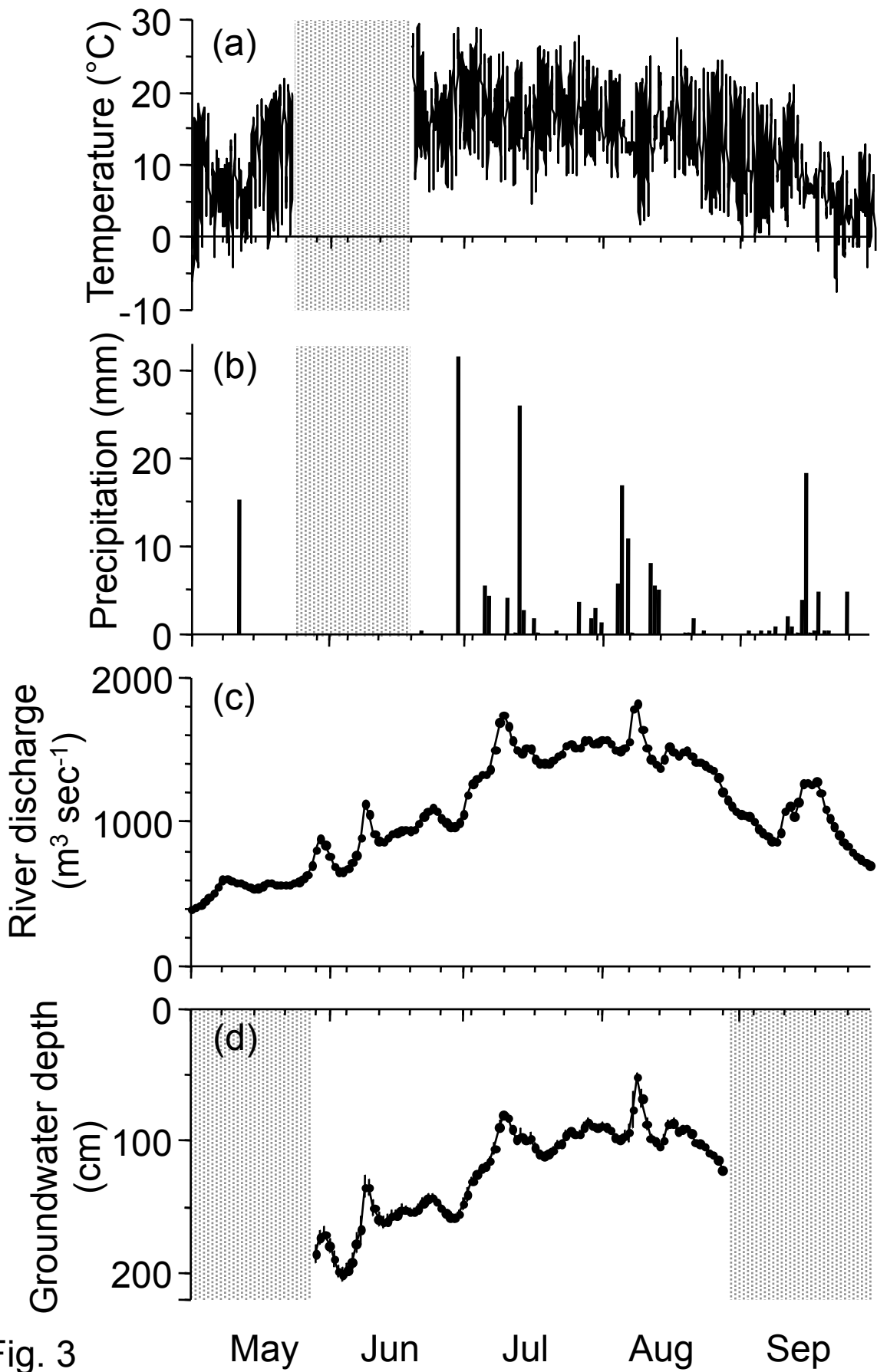


Fig. 3

May

Jun

Jul

Aug

Sep

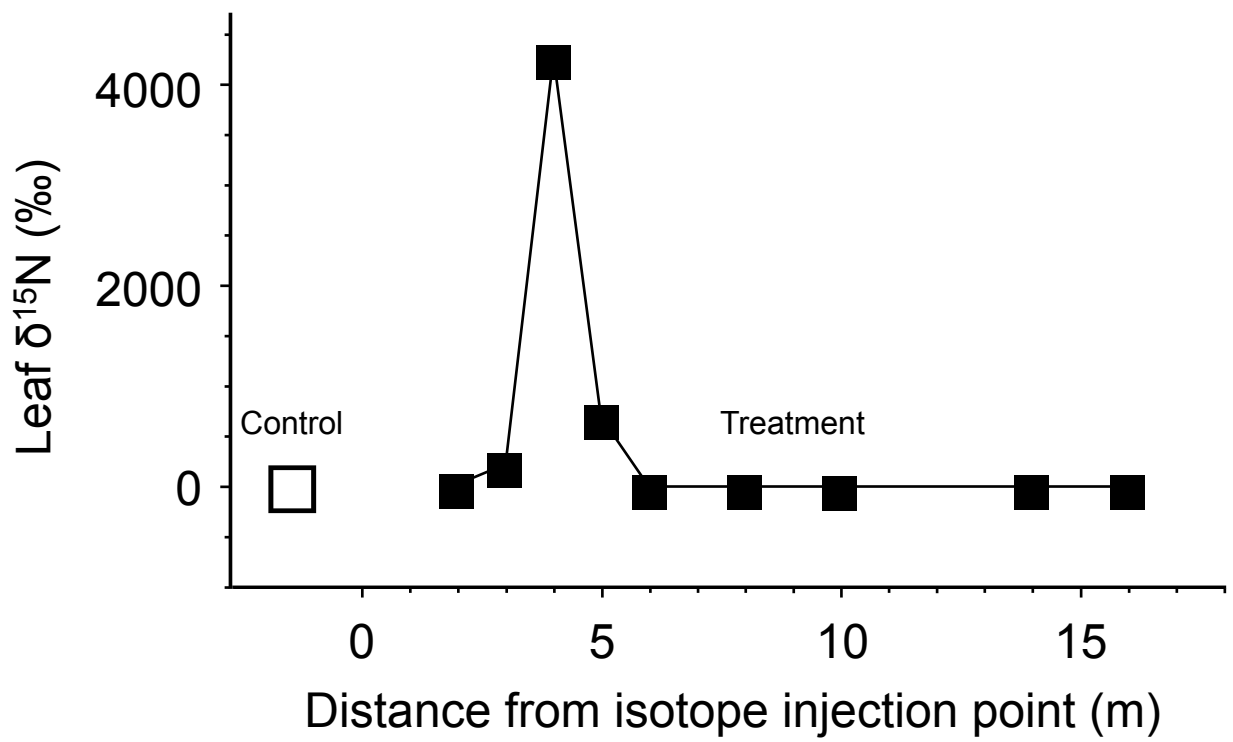


Fig. 4

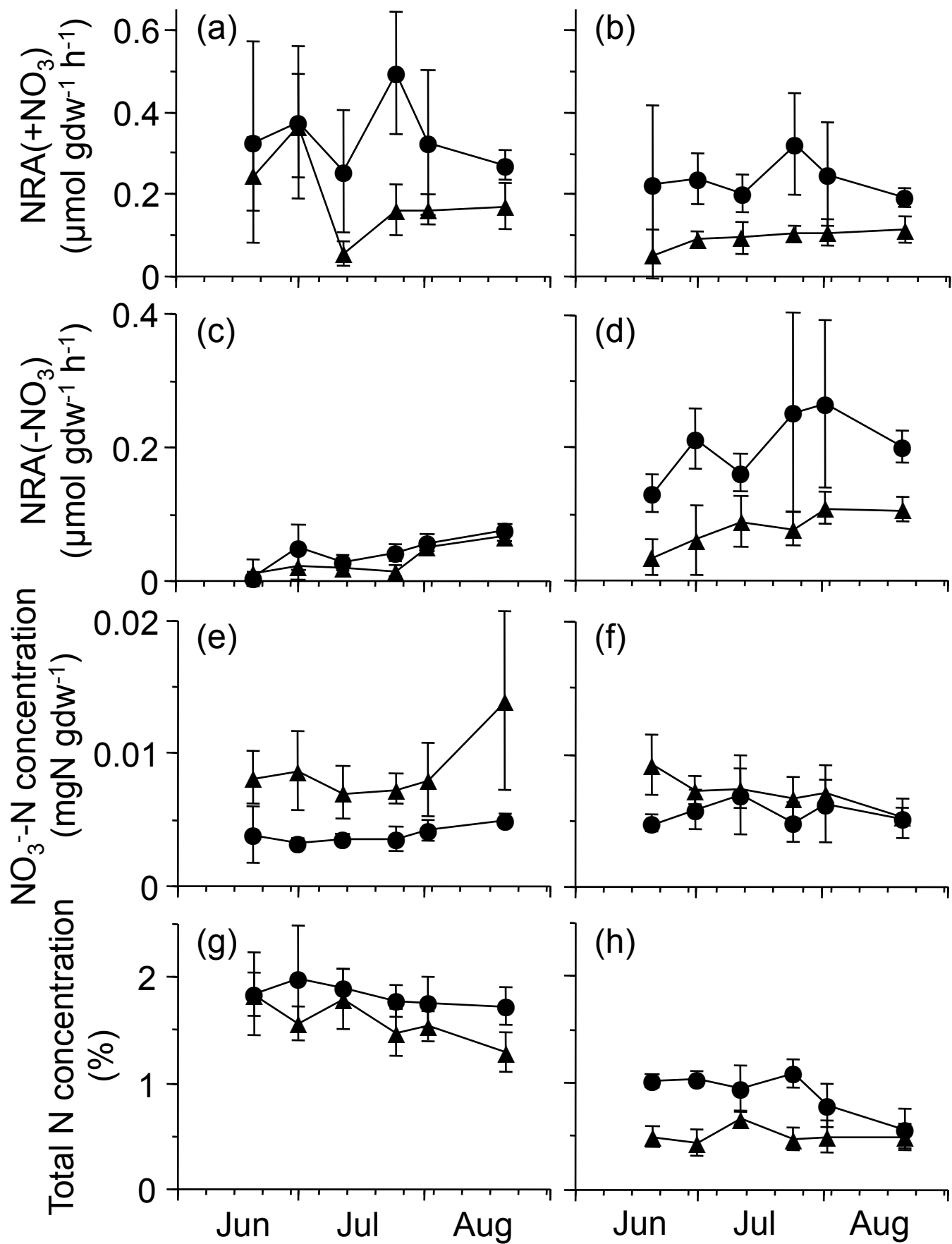


Fig. 5

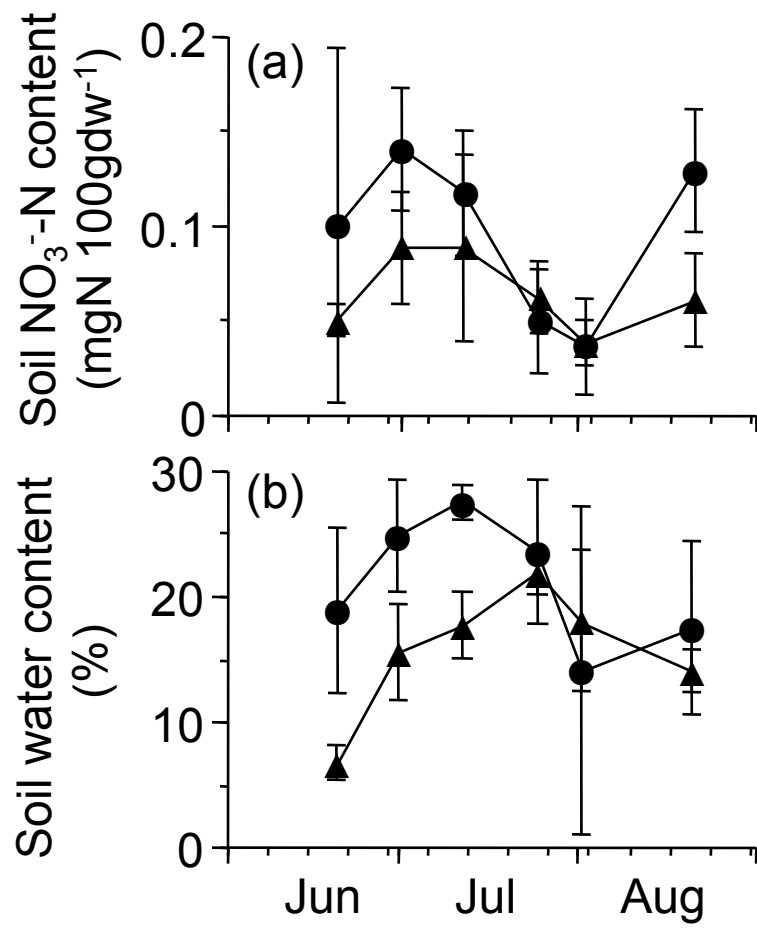


Fig. 6

Table 1 Results of the two-way ANOVA. For plants, NRA(+NO₃), NRA (-NO₃), NO₃⁻-N concentration, and N concentration were compared between species and sampling dates. For soil samples, NO₃⁻-N content and water content were compared between species and among 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	0.11	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