Plant, Cell and Environment (1998) **21,** 1039–1046

Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine

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ABSTRACT

$^{13}NO_3^-$ influx into the roots and *in vivo* nitrate reductase activity (NRA) in the roots and leaves have been measured in trembling aspen (*Populus tremuloides* Michx.) and lodgepole pine (*Pinus contorta* Dougl.) seedlings after exposure to either 0·1 or 1·5 mol m^{-3} NO_3^- for varying periods up to 20 d. Both NO_3^- influx and NRA were inducible in these species and, in trembling aspen, peak induction of nitrate influx and NRA were achieved within 12 h, compared to 2–4 d for influx and 4–12 d for NRA in lodgepole pine. In trembling aspen, ≈ 30% of the total ^{13}N absorbed during a 10 min influx period followed by 2 min of desorption was translocated to the shoot. In lodgepole pine, by contrast, translocation of ^{13}N to the shoot was undetectable during the same time period. Root NRA as well as NO_3^- influx from 0·1 mol m^{-3} NO_3^- were substantially higher in trembling aspen than in lodgepole pine at all stages of NO_3^- exposure, i.e. during the uninduced, the peak induction, and steady-state stages. In order to examine whether the lower rates of NO_3^- influx and NRA were related to proportionately fewer young (unsuberized) roots in lodgepole pine, we determined these parameters in young and old (suberized) roots of this species separately. Induction of influx and NRA were initially greater in young roots but at steady-state there were only minor differences between the young and the old roots. However, even the elevated initial rates in the young roots of lodgepole pine were substantially lower than those of aspen. In pine, influx at 1·5 mol m^{-3} NO_3^- was ~ 6-fold higher than at 0·1 mol m^{-3} NO_3^- and appeared to be mostly via a constitutive system. By contrast, in aspen, steady-state influxes at 0·1 and 1·5 mol m^{-3} were not significantly different, being similar to the rate attained by pine at only the higher $[NO_3^-]$. In aspen, leaf NRA was ~ 2-fold higher than that of roots. In lodgepole pine NRA of the needles was below the detection limit. These results show that trembling aspen seedlings are better adapted for NO_3^- acquisition and utilization than lodgepole pine seedlings.

Keywords: induction; lodgepole pine; nitrate influx; nitrate reductase; nitrogen nutrition; trembling aspen.

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INTRODUCTION

In soils, nitrogen is available as dissolved organic nitrogen (e.g. amino acids), NH_4^+, NO_3^-, or any combination of these, for absorption by plants (Glass & Siddiqi 1995). The relative amounts of these various forms of N depend upon a number of biotic and abiotic factors which determine the rates of mineralization, nitrification, denitrification, leaching, relative uptake of different N forms by plants and microorganisms, etc. (Stark & Hart 1997; Eviner & Chapin 1997). In temperate forest ecosystems, soils under undisturbed coniferous communities are typically high in NH_4^+ and low in NO_3^- (Van Cleve *et al*. 1983; Chapin, Van Cleve & Tyron 1986; Lavoie, Vezina & Margolis 1992). At these sites, even when gross nitrification rates are substantial, soil microbes may rapidly assimilate most of the NO_3^- produced, making it unavailable for plants (Stark & Hart 1997). Following disturbance, characteristically, nitrification rates and soil nitrate concentration ($[NO_3^-]$) increase substantially (Likens, Borman & Johnson 1969; Rice & Pancholy 1972; Lodhi 1978; Vitousek & Melillo 1979; Van Cleve *et al*. 1983; Walley, Van Kessel & Pennock 1996; Prescott 1997). In some cases, soil ammonium concentration ($[NH_4^+]$) may also increase at these disturbed sites if mineralization exceeds nitrification (e.g. Schmidt, MacDonald & Rothwell 1996).

Plant species differ in their capacities for acquisition and assimilation of particular N forms, which is reflected in their natural distribution (Bledsoe & Rygiewicz 1986; Chapin, Moilanen & Kielland 1993; Kielland 1994). For example, it has been reported that many conifers (e.g. white spruce, lodgepole pine, and western hemlock) grow significantly faster when NH_4^+, rather than NO_3^-, is provided as the sole source of N, whereas for others (e.g. western redcedar and Douglas fir), the reverse is true (van den Driessche 1971; Krajina, Maddock-Jones & Mellor 1973; see also Bledsoe & Rygiewicz 1986; Knoepp, Turner & Tingey 1993; Kronzucker, Siddiqi & Glass 1997). A comparative study of the utilization of NH_4^+ versus NO_3^- showed that white spruce had a distinct preference for NH_4^+ as a N source (Kronzucker, Glass & Siddiqi 1995a; Kronzucker, Siddiqi & Glass 1995b,c,d, 1996). It was suggested that a limited capacity for the absorption and assimilation of NO_3^- in white spruce and other late-successional conifers may be an important factor in determining regeneration success at disturbed sites (Kronzucker *et al*. 1997). Implicit in this hypothesis is

the notion that species which more readily colonize disturbed forest sites have superior physiological and biochemical traits for the utilization of NO_3^- as a N source. To evaluate these assumptions, we have undertaken a comparative study of various aspects of uptake, accumulation and assimilation of NO_3^- and NH_4^+ in two early-successional species: trembling aspen (*Populus tremuloides* Michx.) and lodgepole pine *(Pinus contorta* Dougl. var. *latifolia*). The former represents typical fast growing deciduous hardwoods while the latter is a relatively slow growing conifer. In the present paper we describe the patterns of induction of NO_3^- influx and nitrate reductase activity (NRA) in these species.

MATERIALS AND METHODS

Plant culture

Trembling aspen (seedlot 42307) and lodgepole pine (seedlot 3847) seeds were obtained from Tree Seed Centre, Ministry of Forests, Surrey, B.C., Canada. Seeds were sown in a peat/perlite (2: 1 by volume) mixture, containing 4·3 kg m^{-3} dolomite, in styroblocks. Seedlings were maintained in this medium in an outdoor nursery or a greenhouse for a minimum of 3 months. Subsequently, roots were washed gently under running tap water to remove the medium, and then rinsed with deionized water. Each seedling was then fitted into a Plexiglas disc and placed in a Plexiglas hydroponic tank (40 dm^3 capacity) containing 0·1 strength modified Johnson's nutrient solution (Epstein 1972, p. 39) either without N at all, or containing appropriate [NH_4^+] or [NO_3^-]. The composition of the nutrient solution was as follows: macronutrients (in mol m^{-3}), P_i 0·2, K^+ 0·6, Mg^{2+} 0·1, Ca^{2+} 0·4, and micronutrients (in mmol m^{-3}): Cl^- 5, BO_3^- 2·5, Mn^{2+} 0·2, Zn^{2+} 0·2, Cu^{2+} 0·05, Fe-EDTA 2. Nitrate and NH_4^+ were provided as Ca $(NO_3)_2$ and $(NH_4)_2SO_4$, respectively. Solutions were continuously aerated and mixed by means of immersion circulators (Brinkman, USA). The tanks were maintained in a controlled growth room with 16 h/8 h (light/dark) photoperiod, 70% relative humidity, and 20 ± 2 °C temperature. Light was provided at 250 μmol m^{-2} s^{-1} at plant level by fluorescent tubes with spectral composition similar to sunlight (Vita-Lite, Duro-Test, USA). The plants were maintained in the hydroponic tanks for 3 weeks prior to flux measurements and/or nitrate reductase activity assays (see Kronzucker *et al*. 1995a). Solutions were monitored daily for [K^+] (using an Instrumentation Laboratory Model 443 Flame Photometer), for [NO_3^-] (Cawse 1967) and for [NH_4^+] (Solarzano 1969). Solutions were buffered by adding excess powdered $CaCO_3$ to each tank, and pH was maintained at about 6·4–6·8. Solutions were changed completely every week and 1 d before ^{13}N influx measurement and/or NRA assay.

Time-course of induction of nitrate influx and nitrate reductase activity

For induction of NO_3^- influx and NRA, seedlings, grown in a solution containing no N or 25 mmol m^{-3} NH_4^+, were transferred to a solution containing 0·1 or 1·5 mol m^{-3} NO_3^- for various periods of time (0–20 d). In order to measure $^{13}NO_3^-$ influx or NRA at the same time for all treatments (exposed to NO_3^- for different durations), the initiation of NO_3^- pre-treatment was staggered accordingly.

Production of $^{13}NO_3^-$

Nitrogen-13 was produced by proton irradiation of H_2O at the TRIUMF cyclotron on the campus of the University of British Columbia, Vancouver, Canada. The protocol for removal of radiocontaminants was essentially the same as described by Kronzucker *et al*. (1995a), except that 5 cm^3 of 100 mmol m^{-3} (instead of 2·5 mmol m^{-3}) $Ca(NO_3)_2$ was used to elute residual $^{13}NO_3^-$ from the SEP-PAC Alumina-N cartridge. We routinely determined the $t_{1/2}$ of the purified samples which corresponded exactly with that of ^{13}N (9·98 min).

Measurement of influx

Influx of NO_3^- was measured from modified 0·1 strength Johnson's nutrient solution containing appropriate concentrations of ^{13}N-labelled NO_3^-. Seedlings were transferred from hydroponic tanks to a nonradioactive prewash solution, identical in composition (except for isotope) to the uptake solution, for 5 min; they were then transferred to ^{13}N-labelled uptake solution for 10 min. Immediately following loading, roots were dipped into a non-labelled solution for 5 s, and then transferred to a non-radioactive solution, which was otherwise identical to the uptake solution, for 2 min, to desorb $^{13}NO_3^-$ contained in the free space (Kronzucker *et al*. 1995b). Following desorption, roots were excised from shoots, spun for 45 s in a low-speed centrifuge to remove surface solution, and then fresh weights were determined. The radioactivities of shoots and roots were measured with a Packard-γ-counter (Minaxi, Auto-gamma 5000). NO_3^- fluxes thus determined are expressed as (μmol g^{-1} root fresh weight h^{-1}. In determining influx into young (unsuberized) and old (suberized) roots separately, essentially the same procedure was followed as described above except that excised roots were used in these experiments.

Nitrate reductase activity assay

In vivo root NRA assay was carried out using the method described by King, Siddiqi & Glass (1992). Fresh root tissue (0·2–0·5 g) was added to a 10 cm^3 test tube containing 4·25 cm^3 of 100 mmol m^{-3} K-PO_4 buffer (pH 7·7), 0·25 cm^3 isopropanol (99%) and 0·5 cm^3 of 0·5 kmol m^{-3} KNO_3 (final concentration 50 mol m^{-3}). The tubes were capped and purged for 10 min with N_2 which was introduced through the cap via a 25 gauge/3·5 inch spinal needle. After purging, tubes were incubated for 30 min at 30 °C in a water bath. They were then boiled for 10 min to extract tissue NO_2^-. The solution samples were filtered and 1·5 cm^3 aliquots were withdrawn and assayed for NO_2^-

(see below). Nitrate reductase activity was expressed as μmol NO_2^- g^{-1} root fresh weight h^{-1}.

The youngest fully expanded leaves were sampled from aspen and lodgepole pine for *in vivo* NRA assays. The same method as described above for the root NRA assay was used, except that leaf tissue was vacuum-infiltrated for 10 min with NO_3^- solution prior to N_2 purging.

Tissue NO_3^- and NO_2 analysis

Root NO_3^- was extracted by boiling ≈ 0·5 g tissue in 5 cm^3 distilled water for 10 min. The extract was passed through a column packed with insoluble PVPP (polyvinylpolypyrrolidone) to remove phenols and debris. Nitrate was determined spectrophotometrically by the method of Cataldo *et al*. (1975). The sample (0·05 cm^3) was added to 0·2 cm^3 of 50 kg m^{-3} salicylic acid (dissolved in concentrated H_2SO_4), mixed and incubated for 20 min at room temperature. Then, 4·75 cm^3 of 2N NaOH were added and NO_3^- concentration was determined spectrophotometrically by measuring absorbance at 410 nm in a spectrophotometer (Philips, PU2488 UV/VIS).

Nitrite concentration was determined spectrophotometrically, as described by King *et al*. (1992). In brief, 0·25 cm^3 of 0·8 kg m^{-3} *n*-1-naphthylene-diamine-dihydrochloride and 0·5 cm^3 of 20 kg m^{-3} sulphanilamide (dissolved in 5 kmol m^{-3} HCl) were added to 1·5 cm^3 of sample in a 10 cm^3 test tube and mixed. The sample was incubated at room temperature for 30 min, and absorbance was measured at 540 nm.

RESULTS

Nitrate influx

In both trembling aspen and lodgepole pine, NO_3^- influx by the high-affinity transport system (HATS) was inducible. In trembling aspen, induction was very rapid. Within 6–12 h of exposure to 0·1 or 1·5 mol m^{-3} NO_3^-, influx, measured at 0·1 mol m^{-3} NO_3^-, had increased 10–20-fold, from ≈ 0·2 μmol g^{-1} h^{-1} in uninduced plants (constitutive level) to ≈ 2 and 4·3 μmol g^{-1} h^{-1}, respectively (Fig. 1a, inset). Subsequently, NO_3^- influx gradually decreased with increasing duration of exposure to NO_3^- at either [NO_3^-] until 3–4 d, after which influx remained essentially unchanged for up to 20 d (Fig. 1a). However, even this steady-state NO_3^- influx (≈ 0·8–1·2 μmol g^{-1} h^{-1}) was several-fold higher than the constitutive influx.

In lodgepole pine pre-treated with 0·1 or 1·5 mol m^{-3} NO_3^-, induction of NO_3^- influx from 0·1 mol m^{-3} [NO_3^-] required at least 2 d to fully induce this process, and the influx measured at 0·1 mol m^{-3} NO_3^- increased only 2- to 3-fold, from 0·1 μmol g^{-1} h^{-1} (constitutive influx) to about 0·2–0·3 μmol g^{-1} h^{-1}, at either pre-treatment (Fig. 1b). In this species the decline of NO_3^- influx (down-regulation), so evident in aspen, was not apparent even after 20 d of pre-treatment with either external [NO_3^-]. Nitrate influx into roots of lodgepole pine was much lower than in trembling aspen at all stages of NO_3^- pre-treatment. Influx was also measured at 1·5 mol m^{-3} NO_3^- in lodgepole pine, pre-treated with 1·5 mol m^{-3} NO_3^-, and found to be much higher (≈ 6-fold) than at 0·1 mol m^{-3} NO_3^-. Further, when influx was measured at 1·5 mol m^{-3} NO_3^-, neither induction nor down-regulation of NO_3^- influx were evident (Fig. 2).

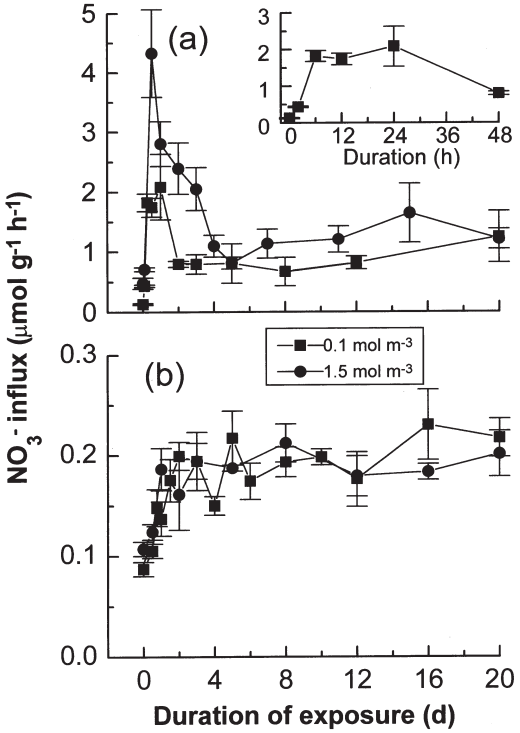

Figure 1. $^{13}NO_3^-$ influx (μmol g^{-1} h^{-1} ± SE) from 0·1 mol m^{-3} NO_3^- into intact roots of (a) trembling aspen and (b) lodgepole pine following pre-treatment with 0·1 or 1·5 mol m^{-3} NO_3^- for 0–20 d (see text). Inset: data for exposures to 0–48 h in (a) redrawn.

Translocation of ^{13}N to the shoot

In trembling aspen pre-treated with 0·1 mol m^{-3} NO_3^- for periods ranging from 0 to 20 d, ^{13}N translocation to the shoot during 12 min of exposure to the isotope (10 min of ^{13}N loading and 2 min desorption) increased as the duration of pre-treatment was extended (Fig. 3). Translocation, expressed as a percentage of the total ^{13}N absorbed, increased from 2% (0 d pre-treatment) to 35% (20 d pre-treatment). Plants pre-treated with 1·5 mol m^{-3} NO_3^- showed a similar pattern (data not shown). In pine, ^{13}N translocation to the shoot was not detectable.

Nitrate reductase activity in the roots and leaves

In trembling aspen, NRA in the roots was fully induced within 6–12 h of exposure to 0·1 or 1·5 mol m^{-3} NO_3^-. NRA increased from ≈ 0·5 μmol NO_2^- g^{-1} h^{-1} in uninduced roots to ≈ 1·6 μmol NO_2^- g^{-1} h^{-1} (Fig. 4a, inset). However,

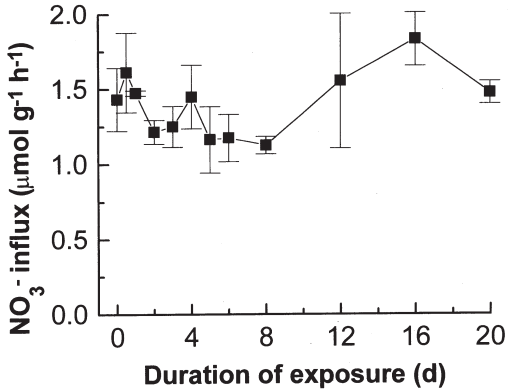

Figure 2. $^{13}NO_3^-$ influx (μmol g^{-1} h^{-1} ± SE) from 1·5 mol m^{-3} NO_3^- into intact roots of lodgepole pine following pre-treatment with 1·5 mol m^{-3} NO_3^- for 0–20 d (see text).

in contrast to the pattern of NO_3^- influx, NRA failed to decline at longer exposures and remained at the maximum level under steady-state conditions. There were no significant differences in the rates of induction or NRA between exposures to 0·1 and 1·5 mol m^{-3} NO_3^- (Fig. 4a). In lodgepole pine, root NRA was lower and its induction slower than that of aspen (Fig. 4b). The peak induction was achieved after 8–12 d of exposure to NO_3^- and the rates of NRA were dependent on pre-treatment concentration: the rate increased from ≈ 0·02 μmol g^{-1} h^{-1} to ≈ 0·4 μmol g^{-1} h^{-1} following pre-treatment with 0·1 mol m^{-3} NO_3^- and to ≈ 0·8 μmol g^{-1} h^{-1} at 1·5 mol m^{-3} NO_3^-. In the latter pre-treatment, however, NRA declined to ≈ 0·5 μmol g^{-1} h^{-1} after 20 d, whereas at 0·1 mol m^{-3} it remained at the fully induced level (Fig. 4b).

In trembling aspen, leaf NRA, similar to that of roots, was fully induced within 1 d. It increased from ≈ 0·1 μmol NO_2^- g^{-1} h^{-1} (constitutive value) to ≈ 3 μmol NO_2^- g^{-1} h^{-1} after one day of exposure to 0·1 mol m^{-3} NO_3^- (Fig. 5). However, no clear pattern of down-regulation was subsequently evident. In lodgepole pine leaves, by contrast, NRA was not detectable even after 20 d of pre-treatment at either external $[NO_3^-]$.

Nitrate influx and NRA in young and old roots of lodgepole pine

At the time of flux determinations and NRA assays, trembling aspen seedlings had proportionately more young roots (50% or more of the total root mass) than lodgepole pine (10–20%). In selected experiments, we determined NO_3^- influx and NRA in lodgepole pine in young and old roots separately. In the young roots, induction was more rapid and both influx and NRA at peak induction were much higher (≈ 6 and ≈ 3 times, respectively) than in the old roots (Fig. 6a,b). Subsequently, in young roots, influx and NRA declined gradually with increasing duration of exposure to NO_3^-. On the other hand, influx and NRA in the old roots remained at the maximum level so that after 20 d of pre-treatment influx in young roots had declined to only 1·7 times that of old roots, while NRA was actually similar in young and old roots (Fig. 6a,b). Nevertheless, even the higher values of NO_3^- influx and NRA in these young roots of lodgepole pine remained substantially lower (≈ 25 and 50%, respectively, for influx and NRA) than average rates (young plus old roots) for trembling aspen (Figs 1a, 4a).

Nitrate concentration of root cells

In the roots of trembling aspen, NO_3^- accumulation was rapid (Fig. 7a). At 0·1 mol m^{-3} NO_3^-, root $[NO_3^-]$ reached a steady value in 1 d, from ≈ 0·2 μmol g^{-1} FW to about 6 μmol g^{-1} FW. Root $[NO_3^-]$ at 1·5 mol m^{-3} external NO_3^- was higher than at 0·1 mol m^{-3} NO_3^- (Fig. 7a). Lodgepole pine roots did not show any change in internal $[NO_3^-]$ after exposure to either external $[NO_3^-]$ for up to 20 d (Fig. 7b).

DISCUSSION

It is well established that both NO_3^- influx and NRA are substrate-inducible processes. Plants grown in the absence of NO_3^- have low rates of NO_3^- uptake and NRA. Exposure to NO_3^- increases the rates of these processes to some peak values (induction), followed by a gradual decline (down-regulation) until a steady value is attained. While this overall pattern is common among plants, species differ greatly in the time scale over which these events occur as well as in the magnitude of these processes. Crop species such as maize, which have high growth rates and generally have a preference for NO_3^- as a N-source, show maximum induction of NO_3^- influx and NRA within a few hours of exposure to NO_3^- (e.g. Oaks 1994; Glass & Siddiqi 1995 for review). By contrast, slow growing conifers such as white spruce, which prefer NH_4^+, may require several days to achieve peak induction (Kronzucker *et al*. 1995a).

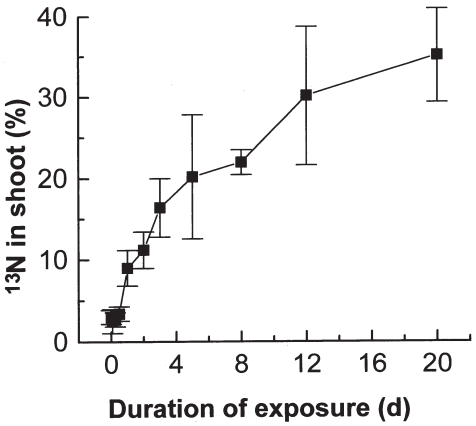

Figure 3. Translocation of ^{13}N to the shoot of trembling aspen (% of total ^{13}N absorbed) during 10 min loading with $^{13}NO_3^-$ followed by 2 min desorption, in plants pre-treated with 0·1 mol m^{-3} NO_3^- for 0–20 d (see text).

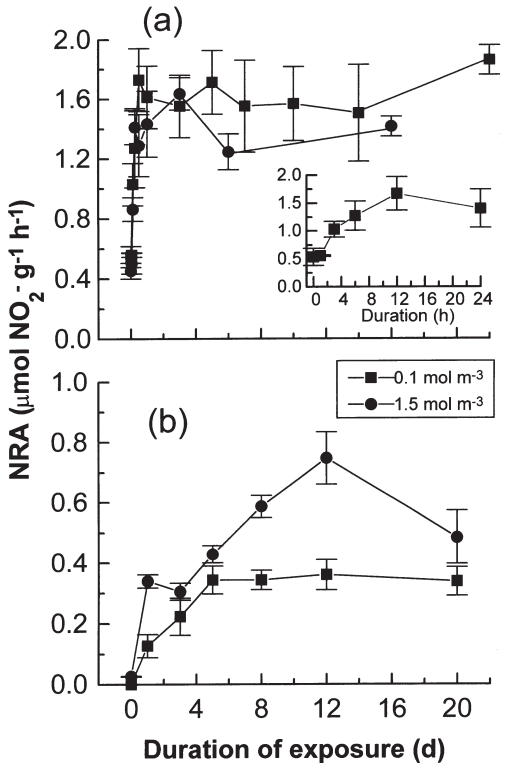

Figure 4. Nitrate reductase activity (NRA) (μmol g^{-1} h^{-1} ± SE) in the roots of (a) trembling aspen and (b) lodgepole pine following pre-treatment with 0·1 or 1·5 mol m^{-3} NO_3^- for 0–22 d (see text). Inset: data for exposures to 0–24 h in (a) redrawn.

In both trembling aspen and lodgepole pine, as in most plant species investigated with respect to this effect, NO_3^- influx as well as NRA were inducible (Figs 1, 4). However, there were substantial differences between these species in the rapidity of induction and the absolute rates of these processes. Trembling aspen appeared to be, by far, the superior species in the utilization of root-available NO_3^-. In this species, induction of both NO_3^- influx and NRA were rapid, with the maximum induction achieved within 6–12 h of exposure to NO_3^-. Lodgepole pine roots, by contrast, required much longer exposure (several days) for maximum induction, which is similar to another conifer, white spruce (Kronzucker *et al*. 1995a). Not only was induction more rapid in trembling aspen, but its rate of NO_3^- uptake (particularly in the range of HATS) and potential rates of NRA were several-fold higher than those of lodgepole pine (Fig. 1,4) and those reported for other conifers (e.g. Kronzucker *et al*. 1995a,bc). Clearly, these differences between the two species are genetic and are not the result of proportionately more young roots in trembling aspen than lodgepole pine at the time of determination of these parameters. Both NO_3^- influx and NRA in the former, determined on a whole-root basis, were much higher than those of young roots of lodgepole pine (Fig. 6).

In trembling aspen, substantial amounts of NO_3^- and/or amino acids were translocated to the shoot; in 0·1 mol m^{-3} pre-treated plants, as much as 35% of the total ^{13}N absorbed was recovered in the shoot within 12 min of exposure to the isotope (10 min $^{13}NO_3^-$ loading followed by 2 min desorption). This, coupled to the high rates of leaf NRA, obviously increases the capacity for utilization of NO_3^- in this species. The rates of leaf NRA we have observed in trembling aspen agree well with those reported for other Populus spp. (Dykstra 1974). By comparison, in lodgepole pine, NRA in the leaves was below the limit of

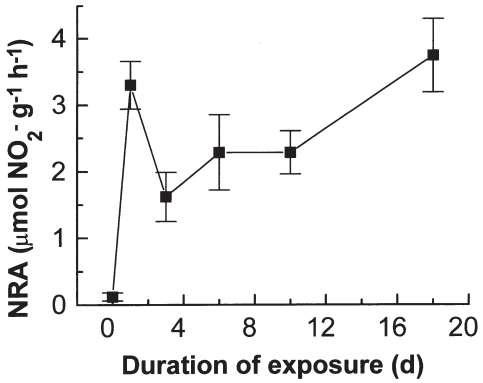

Figure 5. Nitrate reductase activity (NRA) (μmol g^{-1} h^{-1} ± SE) in the leaves of trembling aspen following pre-treatment with 0·1 mol m^{-3} NO_3^- for 0–18 d.

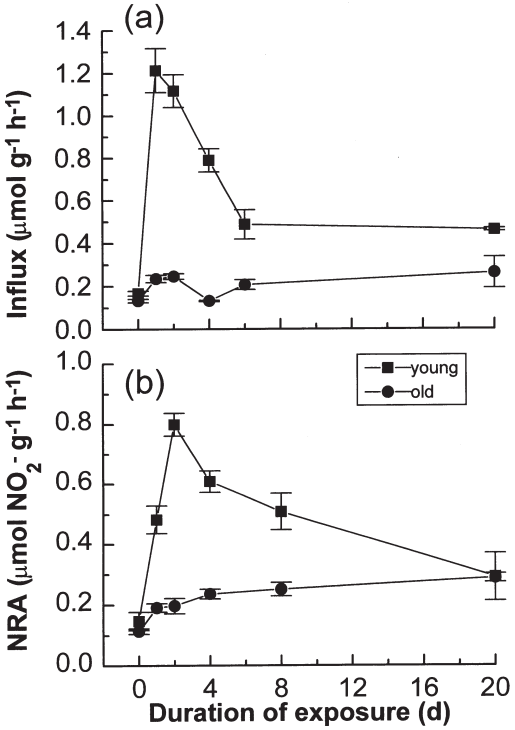

Figure 6. (a) $^{13}NO_3^-$ influx (μmol g^{-1} h^{-1} ± SE) and (b) nitrate reductase activity (NRA) (μmol g^{-1} h^{-1} ± SE) in the young and old roots of lodgepole pine following pre-treatment with 0·1 mol m^{-3} NO_3^- for 0–20 d (see text).

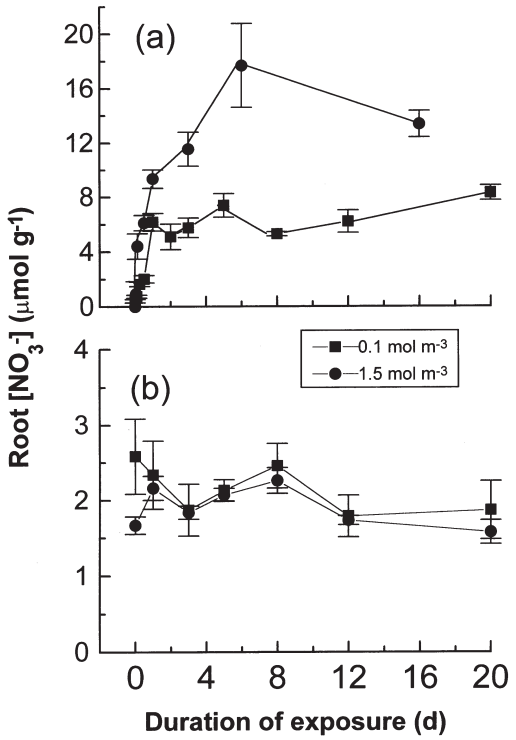

Figure 7. Root [NO_3^-] (μmol g^{-1} h^{-1} ± SE) in the roots of (a) trembling aspen and (b) lodgepole pine.

detection. This is in agreement with the results reported for red spruce (Yandow & Klein 1986). In fact, conifers in general appear to have very low leaf NRA (Smirnoff & Stewart 1985). In some conifers, detectable NRA has been found in the needles, but it was much lower than the root NRA (e.g. Peuke & Tischner 1991). Indeed, it is generally recognized that in conifers most of the NO_3^- is reduced within the roots (e.g. Sarjala 1991 and references therein). This is consistent with the fact that, in lodgepole pine, ^{13}N was not detectable in shoots. Even in experiments which involved exposures to $^{13}NO_3^-$ for 35 min followed by 22 min desorption, only traces of the isotope were found in shoots (our unpublished results). Translocation of N to the shoot may be as NO_3^- and/or as amino acids; generally very little NH_4^+ *per se* is translocated to the shoot (Wang *et al*. 1993; Kronzucker *et al*. 1995d; Glass & Siddiqi 1995). It follows then that in conifers either rates of translocation to the shoot are low not only for NO_3^- but also for amino acids or there is a substantial delay in the appearance of the isotope in the shoot.

Clearly, translocation of ^{13}N to the shoot of trembling aspen was 'inducible' (see Siddiqi, Glass & Ruth 1991; Kronzucker *et al*. 1995a; D. Zhou *et al*. unpublished results); it increased gradually from ≈ 2% in uninduced plants to ≈ 35% after 20 d of pre-treatment (Fig. 2). The rapidity and the magnitude of induction of leaf NRA suggest that a substantial amount of NO_3^- *per se* was translocated to the shoot since NO_3^- itself is the known effector of induction of the NR enzyme. It has been demonstrated that translocation of NO_3^- as well as the enzymes responsible for its metabolism leading to amino acids, i.e. NR, NiR, GS, GOGAT, are all inducible (e.g. Oaks 1994 for review). However, the timescale for induction of these processes (a few hours) is much too rapid to explain the observed pattern of ^{13}N translocation to the shoot. It appears that filling of vacuoles in root cells takes precedence (Glass 1978; Kronzucker *et al*. 1995a) and as the [NO_3^-] in the vacuole increases, flux to the vacuole declines and proportionately more incoming NO_3^- becomes available for translocation to the shoot as well as for assimilation (Siddiqi *et al*. 1990). The half-life of exchange for vacuolar NO_3^- in some crop species has been reported to be ≈ 16–21 h (Lee & Clarkson 1986), which broadly corresponds to the pattern we have observed (Fig. 3).

Interestingly, in lodgepole pine, when influx was measured from 1·5 mol m^{-3} [NO_3^-], induction was not evident, i.e. influx remained unchanged following pre-treatment with 1·5 mol m^{-3} NO_3^- (Fig. 2). In this species, particularly in uninduced plants, the activity of a low-affinity transport system (LATS) was evident at relatively low (0·1–0·2 mol m^{-3}) external [NO_3^-] (our unpublished results). At 1·5 mol m^{-3} NO_3^-, the contribution of LATS, which is constitutive (Siddiqi *et al*. 1990; Kronzucker *et al*. 1995b), may have been so large as to mask changes of HATS activity. Indeed, influx from 1·5 mol m^{-3} NO_3^- was several-fold higher than the V_{max} for HATS (our unpublished results), irrespective of the duration of pre-treatment of lodgepole pine roots with NO_3^-. Thus, the capacity for NO_3^- influx in lodgepole pine roots increased substantially as external [NO_3^-] increased. In trembling aspen, by contrast, steady-state influxes from 0·1 mol m^{-3} and 1·5 mol m^{-3} were similar (0·94 ± 0·06 and 1·33 ± 0·09μmol g^{-1} h^{-1}, respectively) (our unpublished results).

In trembling aspen, NO_3^- influx showed typical negative-feedback effects as a result of prolonged exposures to NO_3^- (Fig. 1, see Glass & Siddiqi 1995 for review). These effects were absent from old lodgepole pine roots. In neither species, however, was influx correlated with root [NO_3^-] or pre-treatment [NO_3^-]. There is evidence at both physiological and molecular levels, that the main effector(s) of negative feedback inhibition is/are some metabolite(s) downstream from NH_4^+, e.g. glutamine, and/or glutamate, rather than NO_3^- *per se* (Lee & Rudge 1986; Lee *et al*. 1992; D. Zhou *et al*. unpublished results). It is noteworthy that even the down-regulated, steady-state NO_3^- influx into aspen roots was ≈ 4-fold greater than that into lodgepole pine roots (Fig. 1). In the latter species, there was no clear indication of down-regulation of influx; in fact, influx slightly increased after 16–20 d of exposure to NO_3^- (Fig. 1b). We speculate that this time-dependent pattern of influx may be the result of increased growth rate at this stage. We have noticed a marked increase in the production of young roots after ≈ 12–16 d of exposure to NO_3^-. Also, in contrast to trembling aspen, there was no accumulation of NO_3^- in the roots of lodgepole pine after exposure to 0·1 or 1·5 mol m^{-3} NO_3^- for as long as 20 d (Fig. 7).

In trembling aspen, potential NRA showed no evidence of negative-feedback effects after prolonged exposures to

NO_3^-, and there was no significant difference in the NRA between the two levels of NO_3^- pre-treatments. In lodgepole pine, by contrast, potential NRA showed evidence of negative feedback inhibition (Figs 4b, 6b). Interestingly, in 0·1 mol m^{-3}-pre-treated lodgepole pine seedlings, NO_3^- influx as well as NRA were induced to much higher levels in the young roots (Fig. 6). However, in these same young roots, both influx and NRA were down-regulated after prolonged exposures to NO_3^- and, at steady state, there were only minor differences between the young and the old roots. Note that the peak influx and NRA levels are similar in young roots pre-treated with 0·1 mol m^{-3} NO_3^- (Fig. 6a,b) to those measured in whole roots (≈ 80–90% old roots) pre-treated with 1·5 mol m^{-3} NO_3^- (Figs 2, 4b). Thus, these observations suggest that 0·1 mol m^{-3} NO_3^- was not adequate to fully induce NO_3^- influx and NRA in the old roots. While root $[NO_3^-]$ determined on a whole-root basis (≈ 80–90% old roots) was similar between the two levels of NO_3^- pre-treatment, cytoplasmic $[NO_3^-]$ was ≈ 20-fold higher in plants pre-treated with 1·5 mol m^{-3} than in those pre-treated with 0·1 mol m^{-3} (our unpublished results; see also Kronzucker *et al*. 1995c for white spruce). It is possible that in the young roots of 0·1 mol m^{-3}-pre-treated plants, cytoplasmic $[NO_3^-]$ was higher, perhaps comparable to the 1·5 mol m^{-3}-pre-treated plants. Indeed, variations in tissue $[NO_3^-]$, NRA and transport activity have been proposed both transversely and longitudinally along the roots and the leaves (see Siddiqi *et al*. 1991 and references therein). In this regard, it is interesting to find that in trembling aspen NRA (Fig. 3a) and cytoplasmic $[NO_3^-]$ (our unpublished results) were both similar between the two pre-treatments.

In the present study, plants were grown in a hydroponic system so that the composition of the pre-treatment nutrient media could be rigorously controlled. These conditions were not conducive to the growth of mycorrhizal fungi and, consequently, the plants used in this study were non-mycorrhizal (Kronzucker *et al*. 1995a,b,c). Furthermore, the aim of our study was to examine genetic differences in the acquisition and utilization of NO_3^- between the two tree species studied. In the case of certain nutrients, notably P_i, mycorrhizae may enhance the uptake capacity of plants. However, mycorrhizae are reported generally not to have any significant effect on the uptake, accumulation and/or reduction of NO_3^- (Rygiewicz *et al*. 1984; Marschner, Häussling & George 1991; Sarjala 1991; Eltrop & Marschner 1996).

In summary, the present study demonstrates that trembling aspen is highly efficient in the acquisition and metabolism of NO_3^-. These adaptations for NO_3^- utilization may be important for the success of this species as an early colonizer of disturbed sites where available N is predominantly NO_3^-. This applies equally to regeneration from seed or from vegetative organs. Generally, coniferous species with a relatively low capacity for NO_3^- utilization would be at a disadvantage under such conditions. On the other hand, conifers appear to be efficient in utilizing NH_4^+. The adaptive significance of a high capacity for NH_4^+ utilization in the late successional or so called 'climax' species (e.g. white spruce) is apparent: soils under these communities are typically high in NH_4^+ and low in NO_3^- (Kronzucker *et al*. 1997 for review). Lodgepole pine, however, is an early successional species, particularly adapted to colonize forest sites following wildfire. Clearly, at low external $[NO_3^-]$ its limited capacity to utilize NO_3^- might place lodgepole pine at a considerable disadvantage in competition with nitrophiles such as aspen. However, at high external $[NO_3^-]$ the capacity of lodgepole pine for NO_3^- utilization increases substantially; in fact, at 1·5 mol m^{-3} NO_3^- its rate of NO_3^- uptake was similar to that of trembling aspen.

ACKNOWLEDGMENTS

We wish to thank the following: Dev Britto, Anshuman Kumar, Mamoru Okamoto, John Vidmar and Degen Zhuo, of the Botany Department, UBC for assistance during the experiments involving $^{13}NO_3^-$, and Mike Adam, Tamara Hurtado and Tom Ruth of TRIUMF, for production of the isotope. $^{13}NO_3^-$ was provided as a gift by TRIUMF. Financial support to R.D.G. and A.D.M.G. from Forest Renewal British Columbia (FRBC) is gratefully acknowledged.

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Received 15 January 1998; received in revised form 20 April 1998; accepted for publication 20 April 1998