Conifer root discrimination against soil nitrate and the ecology of forest succession

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The high incidence of failure when late-successional conifer species are replanted on disturbed forest sites is a considerable problem[1–3]. Here we advance a hypothesis that might explain many of these reforestation problems on a physiological basis, within the framework of forest succession. It is known that the chemical speciation of inorganic nitrogen in forest soils changes from predominantly ammonium (NH_4^+) in late-successional (mature forest) soils to mostly nitrate (NO_3^-) after disturbances such as clearcut harvesting[2–6]. The capacity of plant roots to take up and use these two sources of nitrogen is therefore very important for species establishment on successionally different sites. We have used kinetic and compartmental-analysis techniques with the radiotracer ^{13}N to compare the efficiency of nitrogen acquisition from NH_4^+ and NO_3^- sources in seedlings of white spruce, an important late-successional conifer. We found that uptake of NH_4^+ was up to 20 times greater than that of NO_3^- from equimolar solution, cytoplasmic concentration of NH_4^+ was up to 10 times greater than that of NO_3^-, and physiological processing of NO_3^- was much less than that of NH_4^+. This reduced capacity to use NO_3^- is thought to present a critical impediment to seedling establishment on disturbed sites, where species better adapted to NO_3^- would have a significant competitive advantage.

Inorganic nitrogen is available to plants in soil solution either as NO_3^- or as NH_4^+. Physiological competition at the root level for this resource may have profound effects on relative species performance in the field[7]. Most agricultural species and species confined to poor-quality ruderal or pioneer soils can use either nitrogen source[8]. The ratio of uptake of NO_3^- to NH_4^+ in these species is typically close to 1 (refs 9, 10). Nevertheless, growth on NO_3^- is frequently superior to growth on NH_4^+ (ref. 11), and, on NH_4^+, toxicity is sometimes observed[12]. For this reason, such species are sometimes said to be 'nitrophilous'[2]. Another category of species, including rice[13], ericaceous species and many conifers[2,12], occur naturally on soils enriched in NH_4^+ and organic nitrogen[2–6,13], and appear not to suffer ammonium toxicity[2,12–15]. Nitrate concentrations in these soils are virtually undetectable as a result of inhibited nitrification[2,4,5,13] and/or preferential microbial acquisition of NO_3^- (ref. 31). From an evolutionary perspective, such species might be expected to perform more efficiently on reduced nitrogen than the aforementioned nitrophiles, and to deal successfully with relatively high external NH_4^+. By contrast, the scarcity of NO_3^- would render efficient NO_3^- assimilation virtually irrelevant. Indeed, conifers are reported to grow much better on NH_4^+ than NO_3^- (refs 2, 16, 17).

In most forest soils, available nitrogen changes markedly with the stage of successional development[3]. After disturbance, soil pH generally rises and a new microbial environment appears, which converts soil nitrogen from predominantly NH_4^+ to mostly NO_3^- (refs 2–5). Late-successional conifers therefore become poor competitors for inorganic nitrogen, and the site becomes dominated by nitrophiles[2,3,18–20]. Factors other than adaptation to nitrogen source, such as sun intolerance[18] and inherently slow growth in conifers[21], are also thought to influence this successional pattern. In boreal, montane and subalpine environments, early-successional species, such as aspen, frequently invade sites formerly dominated by spruce after clearcut harvesting, despite replanting with spruce seedlings. Reforestation failure of this sort is substantial in many parts of North America; in British Columbia alone, more than 1.5 million ha of productive forest lands have been classified as failed replantings[1]. Thus, both economically and ecologically, the identification of a possible physiological determinant of reforestation success is of considerable interest.

We have used the radiotracer ^{13}N to assess the differential capacities for the utilization of NO_3^- and NH_4^+ in seedlings of white spruce, a late-successional conifer. Rates of nitrogen uptake were up to 20 times higher for NH_4^+ than for NO_3^- (refs 22, 23). Although V_{max} values under those conditions were vastly different ($\approx 0.1\,\mu mol\,g^{-1}\,h^{-1}$ versus $\approx 2\,\mu mol\,g^{-1}\,h^{-1}$, respectively), K_m values were very similar (15–20 μM), possibly indicating differences in the numbers of the respective transporter proteins in the

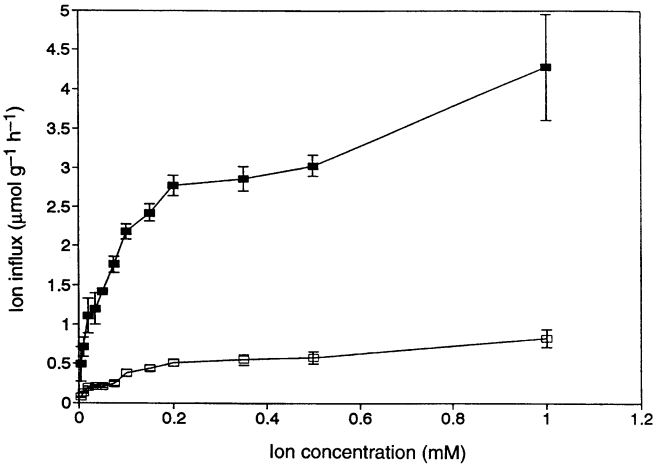

FIG. 1 Comparative concentration dependence of NO_3^- influx (open symbols) and NH_4^+ influx (filled symbols) in roots of intact white-spruce seedlings in the range of environmentally relevant nitrogen concentrations[6]. All plants were pretreated at 100 μM [NO_3] for 3 days. Error bars indicate s.e. ($N \geq 12$).

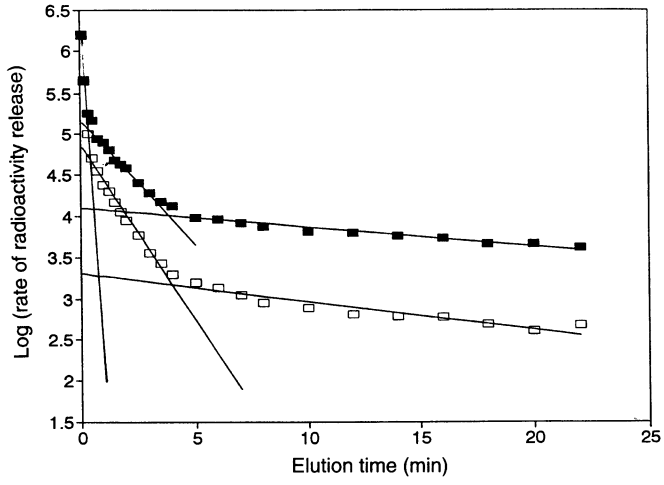

FIG. 2 Combined semi-logarithmic plots of the rate of release of $^{13}NO_3^-$ (open symbols) and $^{13}NH_4^+$ (filled symbols) in [log (c.p.m. released) $g^{-1}\,min^{-1}$] versus time of elution for roots of intact white-spruce seedlings at 100 μM NO_3^- or NH_4^+. Plots include linear regression lines for the three logarithmic phases of efflux for both sets of experiments. Counts eluting from root tissues were corrected for differences in specific activity of the radiotracer to allow for direct comparison of *y*-intercepts.

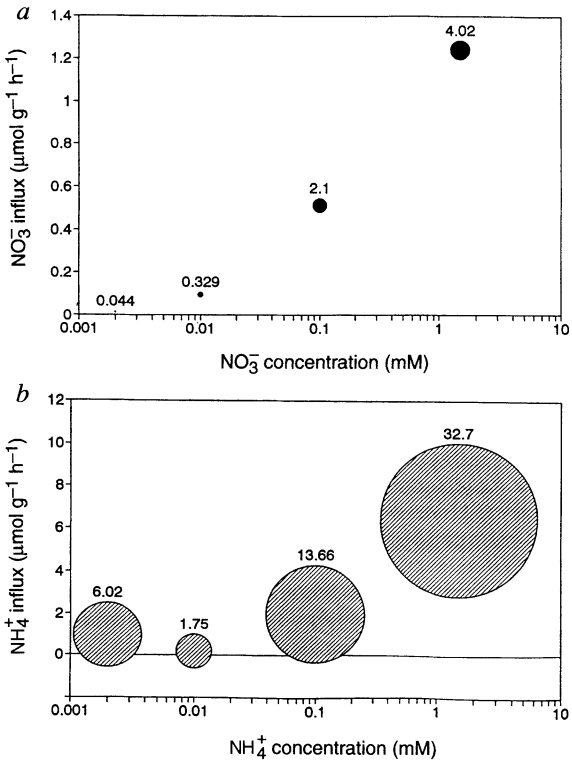

FIG. 3 Comparative plots of influx of *a*, NO_3^- and *b*, NH_4^+ and cytoplasmic pool size at four different external concentrations of the two nitrogen sources. Sizes of shaded circles are calculated to be proportional to one another and represent relative cytoplasmic concentrations (absolute values are indicated (in mM) above). Centres of the circles (*y*-axis values) indicate rates of unidirectional influx of NO_3^- and NH_4^+ under the given conditions as determined by efflux analysis. NO_3^- or NH_4^+ were added 3 days before (and during) the efflux experiment[24,27] (at 10 μM, 100 μM or 1.5 mM). In the case of nitrogen-deprived plants, NO_3^- and NH_4^+ were withheld from growth and pretreatment solutions, and only added (at 10 μM) during labelling and elution to make possible the monitoring of fluxes and the estimations of compartmental concentrations.

plasma membrane, rather than differences in the affinities of the transporters for the two substrates.

As in most species, NO_3^- uptake in spruce is enhanced ('induced') by previous exposure to NO_3^- (ref. 24). However, maximal induction of NO_3^- transport required three days of NO_3^- exposure (at 100 μM NO_3^-), but only several hours in cereals[24]. Indeed aspen, a typical pioneer tree species[12,21], does not require any prior exposure to NO_3^- to achieve maximal uptake (data not shown). This represents further evidence for poor adaptation to utilization of NO_3^- in white spruce. A direct comparison of NO_3^- and NH_4^+ influx in NO_3^--induced seedlings still shows four- to fivefold higher influx for NH_4^+ than for NO_3^- (Fig. 1). A preference of a similar magnitude for NH_4^+ over NO_3^- has been documented in longer-term net-flux estimations in several conifer species[2,14,15,25,26]. Furthermore, spruce also demonstrates low rates of NO_3^- reduction and subsequent metabolic processing[24].

At the subcellular level, efflux analysis was used to determine kinetic constants for the exchange of NO_3^- and NH_4^+, to quantify the extent of efflux under varying nitrogen supply, as well as to estimate the cytoplasmic concentrations of the two ions. Three kinetically distinct subcellular compartments were revealed for both nitrogen sources (Fig. 2): (1) a root-surface film; (2) an adsorptive component of the cell wall; and (3) the root-cell cytoplasm[24,27,28]. Half-lives of exchange for these compartments for NO_3^- were 2 s, 20 s and 7 min, respectively, and for NH_4^+ they were 2 s, 30 s and 14 min, respectively[24,27,28]. Efflux analysis confirmed the strong preference for NH_4^+. Plots of $^{13}NO_3^-$ and $^{13}NH_4^+$ efflux clearly show significantly lower *y*-intercepts for both the cell-wall and the cytoplasmic regression lines for NO_3^- compared to NH_4^+ (Fig. 2). The differences indicate smaller fluxes and a smaller accumulation capacity in the cell-wall free space and the cytoplasm of spruce roots for NO_3^- compared to NH_4^+ (refs 24, 27). We measured the changes in influx and cytoplasmic concentrations of NO_3^- was consistently five- to eightfold lower than that of NH_4^+. In seedlings previously starved of nitrogen, NO_3^- accumulation in the cytoplasm was 140-fold lower, an effect which can be attributed to the fact that NO_3^- uptake requires induction, whereas NH_4^+ uptake does not. Cytoplasmic NH_4^+ in spruce roots was similar in magnitude to levels in rice, which is also adapted to NH_4^+ soils[13], and to NO_3^- levels in agricultural species[24,27], which have superior growth on NO_3^- (refs 11, 12, 18–20).

Mycorrhization of the root system, as would be expected for spruce seedlings in the field, has been shown to enhance NH_4^+ absorption rates in conifers, but does not significantly affect rates of NO_3^- uptake[29,30]. The preference for NH_4^+, as observed in our non-mycorrhizal plant material, should therefore be accentuated even further in the field. In our opinion, given the pronounced inherent differences in the physiological utilization capacities for NH_4^+ and NO_3^- as sources of nitrogen, it is not surprising that reforestation problems are encountered with species such as white spruce on disturbed sites, where NO_3^- is the predominant nitrogen source and NH_4^+ is in short supply. □

Methods

Influx experiments. Three-month-old nursery-grown seedlings of *Picea glauca* (Moench) Voss.[27] were transferred to hydroponic tanks containing aerated 1/10-strength modified nitrogen-free Johnson's solution. Growth and pretreatment conditions were as described[27]. NO_3^- (as $Ca(NO_3)_2$) or NH_4^+ (as $(NH_4)_2SO_4$) were added during uptake at the indicated concentrations. The radiotracer ^{13}N was provided by the Tri-University Meson Facility (TRIUMF) at the University of British Columbia, and $^{13}NH_4^+$ and $^{13}NO_3^-$ were generated[13,27]. Roots of intact seedlings were equilibrated for 5 min in non-labelled solutions chemically identical to the uptake solutions, transferred to uptake vessels containing $^{13}NH_4^+$ or $^{13}NO_3^-$ labelled solution for 10 min, and postwashed in non-labelled solution for 2 min (for NO_3^-) or 3 min (for NH_4^+) to desorb tracer contained in the free space[23,23]. Seedling roots were then excised from shoots, the roots were spun in a low-speed centrifuge for 30 s to remove surface liquid, and the fresh weights of roots and shoots were determined. The radioactivities of roots and shoots were determined in a Packard γ-counter (Minaxi δ, Auto-γ 5000 Series). Using the value for specific activity ($^{13}N/(^{13}N + ^{14}N)$) of the loading solution and the total fresh root weight of each seedling, NO_3^- or NH_4^+ fluxes were calculated and expressed in μmol g^{-1} h^{-1} (refs 22, 23). Experiments were repeated four times. Each experimental treatment consisted of three seedling samples (minimum root mass was 3 g fresh weight per sample). Data from several experiments were pooled ($N \geq 12$) for calculations of means and standard errors. These values were used for plotting the representative concentration-dependent curves, as well as for calculating V_{max} and K_m values as described[22].

Compartmental analysis. Roots of intact seedlings (grown at steady-state provision of 10 μM, 100 μM or 1.5 mM NO_3^- or NH_4^+) were equilibrated in non-labelled preloading solution for 5 min before transfer to the ^{13}N-loading solution. Roots were then immersed in ^{13}N-labelled loading solution for 35 min (for NO_3^-) to 60 min (for NH_4^+) to bring the cytoplasmic phase to a specific ^{13}N activity close to that of the loading solution[27,28]. The seedlings were transferred to efflux funnels[13], and the roots eluted successively with small portions of non-labelled solution for varying times. With $t = 0$ as the time of transfer from loading to washing solution, and $t_{final} = 22$ min for the final elution, the time periods for the 25 successive washes were: 5 s (2×), 10 s (2×), 15 s (6×), 30 s (4×), 1 min (4×), 2 min (7×). The eluates were then counted in a Packard γ-counter as above. Roots were excised from the shoots after the final elution, were spun for 30 s, and plant organs were weighed and counted. Treatment of data was as described[24,27]. Experiments were repeated 4–7 times, with two replicates each. Standard errors for various derived parameters (half-lives, fluxes, pool sizes) were within 15% of the means ($N \geq 8$). Representative experiments were chosen for semi-logarithmic plots of the rate of ^{13}N release versus elution time (Fig. 2). Because the specific activity in the plant compartments during elution is declining exponentially, the logarithm of the rate of release of radioactivity from the plant tissue can be plotted against elution time[13,4,27,28]. Linear regression on the semi-logarithmic plots was then used to resolve separate phases. The slopes of the regression lines, after conversion to natural logarithm, yielded kinetic exchange constants (*k*) for the respective phases, which could be expressed as

half-lives of exchange ($t_{1/2} = 0.693/k$). The intercept with the ordinate of the regression line for the presumed cytoplasmic phase (that is, the rate of ^{13}N release from the slowest-exchanging phase at time zero) indicates the size of the cytoplasmic NO_3^- or NH_4^+ pool[13,28]. Cytoplasmic concentrations of NO_3^- or NH_4^+ were calculated from the quotient of the integrated rate of ^{13}N release during five times the half-life of cytoplasmic exchange and the ratio of efflux to all fluxes removing $^{13}NO_3^-$ or $^{13}NH_4^+$ from the cytoplasm, and assuming 5% for cell volume occupied by the cytoplasm[24,27,28].

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High rates of nitrification and nitrate turnover in undisturbed coniferous forests

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THE importance of nitrate (NO_3^-) in the internal nitrogen cycle of undisturbed coniferous ecosystems has not been widely recognized[1,2]. Nitrate concentrations in soils from these forests tend to be low, and assays measuring net nitrification usually show exceedingly slow rates[3,4]. It may be, however, that microbial assimilation of NO_3^- is substantial in these soils, and that net nitrification rates greatly underestimate gross rates[5]. Here we use a ^{15}N isotope-dilution technique in intact soil cores to measure gross rates of nitrification and microbial assimilation of NO_3^- in eleven undisturbed forest ecosystems of New Mexico and Oregon. We found that gross nitrification rates were surprisingly high in all of the forests examined. Net nitrification rates poorly predicted gross rates because the soil microbial communities had the capacity to assimilate almost all of the NO_3^- produced. To our knowledge, this is the first report of gross nitrification and NO_3^- assimilation rates in intact soil samples from a large number of contrasting forest ecosystems. Our results contradict previous assumptions that nitrification rates are low in mature coniferous forests and suggest that current models greatly underestimate the role of the microbial community in preventing NO_3^- loss.

Studies examining nitrogen retention in forest ecosystems have focused on net nitrification, net mineralization, microbial assimilation of ammonium (NH_4^+), and plant uptake; however, microbial assimilation of NO_3^- has been largely ignored. Early studies showed that soil microbial communities prefer NH_4^+ to NO_3^- as a source of nitrogen[6], and measurable quantities of NH_4^+ are almost always present in soils. Therefore, it has generally been assumed that NH_4^+ will be the nitrogen source for microbes, and that microbial assimilation of NO_3^- will be minimal[7,8].

Microbial assimilation of NO_3^- has been discounted as a significant process controlling NO_3^- pool sizes and as a mechanism of nitrogen retention following disturbance in forest ecosystems[3,9,10], but we considered that substantial amounts of microbial assimilation of NO_3^- might occur in these systems because: high rates of carbon addition to soils are likely to result in nitrogen limitation to the microbial biomass; high spatial variability in carbon inputs is likely to result in microsites of mineralization and nitrification adjacent to microsites of intense immobilization; and fungal populations dominating these soils are capable of translocating NO_3^- from microsites with high mineralization and nitrification to microsites with high carbon availability.

We evaluated the importance of nitrification and microbial assimilation of NO_3^- by measuring rates in eleven forest soils along an elevational transect in the Tesuque watersheds of northern New Mexico[3,9,10] and a latitudinal transect (at about 44° N) extending 220 km from the Oregon coast to the east side of the Cascade Mountains in central Oregon[11,12] (Table 1). The ecosystems along these two transects represent a wide range of forest ecosystems and span almost the entire range of above-ground net primary production that occurs in forests of North America (1 to 13 Mg ha^{-1} yr^{-1})[11,12].

Gross rates of nitrification and NO_3^- consumption were measured in late spring (May and June) and late summer (August) using $^{15}NO_3^-$ isotope dilution[13]. Rates were measured during *in situ* incubation of intact core samples and homogenized samples from the 0–15-cm mineral soil layer. Homogenized samples were incubated with and without acetylene (at 10 kPa): to verify the validity of isotope dilution measurements; to evaluate the relative importance of autotrophic compared with heterotrophic nitrification; and to determine how much NO_3^- consumption was due to denitrification. Rates of microbial assimilation of NO_3^- were also verified by measuring microbial biomass ^{15}N and total soil organic ^{15}N at the end of the incubations.

Gross nitrification rates in intact soil cores were high at all eleven forest sites (Table 2), ranging from 25 mg N m^{-2} d^{-1} in the New Mexico ponderosa pine site during summer, to >300 mg N m^{-2} d^{-1} in the Douglas-fir site during spring. These rates are one to two orders of magnitude higher than rates of nitrogen input from litterfall[3,11], indicating that cycling of NO_3^- through the soil microbial community is extremely rapid relative to plant nitrogen uptake. High nitrification rates occur in these forests in spite of low soil pH (Table 1), low nitrogen deposition rates, and low availability of nitrogen. Wet deposition of nitrogen at each of these sites averaged <2 kg N ha^{-1} yr^{-1} during the past decade[14].

Comparison of nitrification rates determined by isotope dilution in homogenized samples with rates determined by acetylene inhibition verified that rate estimates from isotope dilution are reliable ($k_a = -0.08 + 0.97k_N$, where k_N is the gross nitrification rate determined by ^{15}N isotope dilution (in mg N kg^{-1} d^{-1}), and k_a is the rate calculated from the difference between net NO_3^- consumption with and without acetylene; $r^2 = 0.67$; $n = 34$). Acetylene inhibition also showed that, in spite of low soil pH, almost all of the nitrification was due to the activity of autotrophic