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Opinion paper

Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis

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Ratios of ammonium (NH_4^+) to nitrate (NO_3^-) in soils are known to increase during forest succession. Using evidence from several previous studies, we hypothesize that a malfunction in NH_4^+ transport at the membrane level might limit the persistence of early successional tree species in later seral stages. In those studies, ^{13}N radiotracing was used to determine unidirectional fluxes and pool sizes of NH_4^+ and NO_3^- in seedlings of the late-successional species white spruce (*Picea glauca*) and in the early successional species Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) and trembling aspen (*Populus tremuloides*). At high external NH_4^+, the two early successional species accumulated excessive NH_4^+ in the root cytosol, and exhibited high-velocity, low-efficiency (15% to 22%), membrane fluxes of NH_4^+. In sharp contrast, white spruce had low cytosolic NH_4^+ accumulation, and lower-velocity but much higher-efficiency (65%), NH_4^+ fluxes. Because these divergent responses parallel known differences in tolerance and toxicity to NH_4^+ amongst these species, we propose that they constitute a significant driving force in forest succession, complementing the discrimination against NO_3^- documented in white spruce (Kronzucker et al. 1997).

Introduction

Forest ecosystems evolve over centuries, as organisms and environments exert influences on one another and alter the composition of plant communities (Finegan 1984). Changes in light regimes (Smirnoff and Stewart 1985, Gilbert et al. 2001), moisture and temperature profiles (Clements 1928, Finegan 1984, Bonan 1992), and allelopathic activities (Rice and Pancholy 1972, Lodhi and Killingbeck 1980) are among the most important factors that determine these patterns of forest succession. Because nitrogen limits the growth of forest plants more than any other soil nutrient (Vitousek et al. 1982, Tilman 1986, Vitousek and Howarth 1991), changes in soil N chemistry are also likely to be significant determinants of synecological changes in forests (Lodhi 1977, Lavoie et al. 1992, Eviner and Chapin 1997, Kronzucker et al. 1997). In temperate and boreal forest ecosystems, a substantial increase in soil nitrate (NO_3^-) characteristically follows disturbances such as fire, avalanche clearing, windthrow or clearcut harvesting (Likens et al. 1969, Vitousek et al. 1982, Kronzucker et al. 1995a, b, 1997). Conversely, ammonium (NH_4^+) dominates the relatively undisturbed soils in these zones (Robertson 1982, Blew and Parkinson 1993), to the point of its exclusive presence as an inorganic N source on many sites.

Given the diversity of plant responses to inorganic N sources, such changes must have implications for forest colonization patterns over the longterm. We have previously (Kronzucker et al. 1997) argued that, over the course of their evolution, trees have become adapted to the nitrogenous characteristics of their native environments. A consequence of this specialization, however, is reduced competitive ability on soils of differing nitrogen profile. In the case of the conifer white spruce, which has a widespread distribution and dominance in late-successional stages of temperate and boreal forests

(Farrar 1995), we showed that the exclusion of the species from disturbed, NO_3^--rich, sites is associated with an exceptionally poor, and evidently atrophic, utilization capacity for NO_3^- at the levels of uptake, metabolism, and intracellular storage (Kronzucker et al. 1997). Importantly, these physiological attributes mirror the superior growth of white spruce on NH_4^+ compared to NO_3^- (Kronzucker et al. 1997). By contrast, the growth of many early-successional forest plants and agricultural species is typically superior on NO_3^-, while prolonged exposure to NH_4^+ can cause symptoms of toxicity, death, or even species extirpation (Krajina et al. 1973, Smirnoff and Stewart 1985, Gijsman and de Willigen 1991, Oltshoorn et al. 1991, de Visser and Keltjens 1993, de Graaf et al. 1998, Britto and Kronzucker 2002). Why such plants experience growth inhibition on NH_4^+, given that NH_4^+ is a ubiquitous intermediate in N metabolism, has been the subject of much controversy (Gerendas et al. 1997, Britto and Kronzucker 2002).

In order to discern a more general role of nitrogen in forest succession, it was important to compare the cellular flux and acquisition patterns for inorganic N in white spruce (Kronzucker et al. 1997) with these patterns in early-successional tree species. In a follow-up study (Min et al. 1999) extending the work with white spruce, we chose trembling aspen and Douglas-fir as comparable early-successional model systems, because of their widespread occurrence in the earlier phases of temperate and boreal forest chronosequences (Farrar 1995). Based on cumulative results from these and other studies, we hypothesize in the present paper that differential adaptations in root membrane transport for inorganic N contribute to the exclusion of these early successional species from late-successional sites, as shown for aspen in Fig. 1.

Hypothesis

Net N acquisition by roots

Our hypothesis, that differential adaptations to inorganic N transport in roots can influence patterns of forest colonization, is supported by several lines of evidence involving use of the radiotracer ^{13}N (for details of experimental procedures and analysis, we refer the reader to Kronzucker et al. 1995a, 1995b, Min et al. 1999, and Britto and Kronzucker 2001). Table 1 shows significantly higher rates of net N acquisition of NH_4^+ over NO_3^- in all three species and at both N provision levels, although it must be emphasized that this parameter does not by itself reveal N source preferences. Importantly, however, in the early-successional species, net acquisition of NH_4^+ over and above that of NO_3^- does not translate into a growth advantage, as it does in white spruce, but instead can inhibit growth (Krajina et al. 1973, Smirnoff and Stewart 1985, Gijsman and de Willigen 1991, Oltshoorn et al. 1991, de Visser and Keltjens 1993, Min et al. 1999). We have also observed toxicity responses in aspen and Douglas-fir under the conditions of interest here, using growth trials and measurements of chlorophyll fluorescence in the field, greenhouse, and laboratory (our unpublished results). Clearly, the poor adaptation to NH_4^+ as an N source in these trees is not an outcome of

Fig. 1. Aspen invades a mountainside in the Southern Rocky Mountains, New Mexico, after clear-cut harvesting of a mature stand of spruce. Mature spruce still covers the opposing, undisturbed, mountainside. The onset of fall coloration in aspen highlights its almost exclusive presence post-disturbance and its almost complete absence from the mature, late-successional, forest stand. The present study advances an explanation, based upon root-transport adaptations to soil nitrogen, for the success of aspen on the early-successional site and its exclusion from the late-successional stand.

Table 1. Ratios of NH_4^+ to NO_3^- for influxes and cytosolic pool sizes in the three tree species grown and assayed at 0.1 and 1.5 m*M* external $[NH_4^+]$ or $[NO_3^-]$ (denoted $[N]_o$). Note the exceptionally high values in Douglas-fir and aspen at 1.5 m*M*. Data are from Kronzucker et al. (1995a,b), and Min et al. (1999). ϕ_{net} denotes the net flux of N into the plant; ϕ_{oc} denotes unidirectional influx across the plasma membrane, and $[N]_{cyt}$ denotes the cytosolic concentration of nitrate or ammonium in root cells.

	White spruce		Douglas-fir		Trembling aspen	
$[N]_o$	0.1 m*M*	1.5 m*M*	0.1 m*M*	1.5 m*M*	0.1 m*M*	1.5 m*M*
ϕ_{net} ratio	2.9	4.3	2.9	11.4	2.7	6.5
ϕ_{oc} ratio	3.9	5.2	4.2	28.3	3.2	23.0
$[N]_{cyt}$ ratio	6.8	8.1	3.9	32.6	3.0	23.1

an insufficient uptake capacity for the ion, a situation quite distinct from the atrophic uptake of NO_3^- in white spruce.

Unidirectional N fluxes at the root plasma membrane

On the contrary, examination of unidirectional fluxes of NH_4^+ at the higher N provision level reveals that uptake capacity in the early-successional species is excessive, and insufficiently regulated. Figure 2 shows results from several such unidirectional flux studies (Kronzucker et al. 1995a, 1995b, Min et al. 1999), comparing $^{13}NH_4^+$ release from roots of trembling aspen, Douglas-fir and white spruce at 1.5 m*M* external NH_4^+ ($[NH_4^+]_o$). The tails of the exponential efflux curves shown represent tracer release from the cytosol of root cells (Kronzucker et al. 1995c) (see dotted regression line in Fig. 2), and analysis of this cytosolic efflux allows the determination of unidirectional influx across the root plasma membrane as well as the quantification of pool sizes of N in the root cytosol. Figure 2 illustrates that a substantially higher efflux of NH_4^+ from the roots of the two early-successional species is found in white spruce at this concentration of NH_4^+. An analysis of subcellular partitioning of radiolabelled N (Fig. 3) revealed that the large efflux in the early-successional species was associated with unusually high unidirectional influx across the plasma membranes of root cells (see also Table 1), with only a small fraction of incoming N being channelled to N metabolism, to the shoot, or to root-cell vacuoles. The high percentage of efflux (78–85% of influx, compared to 35% in white spruce) indicates a futile cycling of NH_4^+ at the plasma membrane, i.e. an exceptionally low efficiency [calculated simply from (1-efflux/influx) × 100%] of acquisition for this N source (22% and 15%, versus 65%, respectively). In short, NH_4^+ in these species appears to be lost almost as rapidly as it is gained, which provides evidence for a poor adaptation to this N source, rather than for its preference. The lack of selectivity against NH_4^+ at the membrane level indicates an impairment in the first line of defense plants have evolved to protect against this potentially toxic nutrient (Britto and Kronzucker 2002). Ammonium flux impairment, which must precede (or coincide with) any deleterious effects of NH_4^+ on plant growth, cannot be discerned by net-flux analyses, but only from studies of unidirectional fluxes, and therefore was not observed by

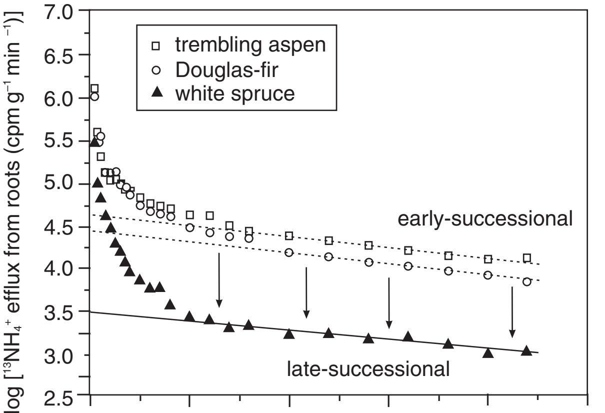

Fig. 2. Semi-logarithmic plots of $^{13}NH_4^+$ efflux from roots of trembling aspen, Douglas-fir, and white spruce at 1.5 m*M* $[NH_4^+]_o$. Linear regressions indicate NH_4^+ efflux from the cytosol of root cells in the three species. Note the similarities in the efflux patterns for aspen and Douglas-fir, and the stark difference (log scale) between the two early-successional species and white spruce. Plots are corrected for differences in root mass and specific activity of the ^{13}N tracer, affording direct comparisons. Arrows indicate a shift in physiological condition from early to late succession. Data are from Kronzucker et al. (1995a, 1995b), and Min et al. (1999).

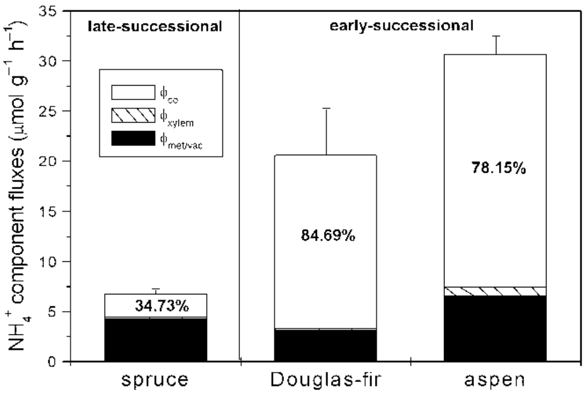

Fig. 3. Subcellular component fluxes of NH_4^+ in root cells of trembling aspen, Douglas-fir, and white spruce exposed to 1.5 m*M* $[NH_4^+]_o$. Filled segments represent combined NH_4^+ flux to metabolism and to the root-cell vacuoles ($\phi_{met/vac}$), shaded segments represent N flux to the shoot (ϕ_{xylem}), and open segments represent NH_4^+ efflux from the roots (ϕ_{co}; also indicated as percentage of influx). The sum of these component fluxes equals influx of NH_4^+ into roots cells (ϕ_{oc}). Standard error bars pertain to ϕ_{oc} (n = 8). Note the high efflux percentage in the early-successional species. Data are from Kronzucker et al. (1995a, 1995b), and Min et al. (1999). For flux symbols, see Table 1.

earlier workers who undertook net-flux studies, in Douglas-fir (Kamminga-van Wijk and Prins 1993), aspen (Chapin et al. 1986), and spruce (Marschner et al. 1991, Gessler et al. 1998).

NH_4^+ cycling, pools, and toxicity

Cytosolic NH_4^+ concentrations were as high as 80 m*M* in Douglas-fir and 130 m*M* in trembling aspen at 1.5 m*M* $[NH_4^+]_o$, while concentrations in white spruce did not exceed 30 m*M* (Fig. 4; see also Table 1). Interestingly, the moderate cytosolic accumulation and the more tightly regulated influx, as evidenced by high flux efficiency, in white spruce were similar to those measured in rice (*Oryza sativa*), a species renowned for its efficient utilization of NH_4^+ (Wang et al. 1993, Britto et al. 2001). By contrast, the elevated cytosolic NH_4^+ concentrations, and the excessive influx coupled to low flux efficiency in Douglas-fir and aspen closely resemble the condition documented in barley (*Hordeum vulgare*), known for its susceptibility to NH_4^+ toxicity (Britto et al. 2001, Britto and Kronzucker 2002). A major effect of uncontrolled cytosolic accumulation of NH_4^+ in sensitive species is likely to be the displacement of other cations, such as K^+, whose homeostatically controlled cytosolic concentration is thought to play a pivotal role in cell function (Walker et al. 1996). Although cytosolic K^+ levels have not yet been documented as a function of $[NH_4^+]_o$, substantial decreases in total-tissue K^+ (and other cations, such as Ca^{2+} and Mg^{2+}) have been shown to be associated with high $[NH_4^+]_o$ in species prone to NH_4^+ toxicity (Gerendas et al. 1997, Britto and Kronzucker 2002). A breakdown of cytosolic concentration homeostasis of key ions such as K^+, brought about by the inability of susceptible plants to exclude NH_4^+, may provide a partial explanation for NH_4^+ toxicity at the cellular level. Further explanation may involve thermodynamic aspects of NH_4^+ fluxes, in particular an energy-demanding efflux of this N source at toxic external concentrations, as has recently been demonstrated in barley (Britto et al. 2001, Kronzucker et al. 2001). It is noteworthy that species as taxonomically distinct as barley, Douglas-fir, and aspen on the one hand, and rice and white spruce on the other, should display cellular behaviours so strikingly similar. This similarity is suggestive that sensitivity or tolerance to high NH_4^+ exposure is strongly determined by primary N-transport characteristics of plant cell membranes, both in agricultural and natural settings (Britto and Kronzucker 2002).

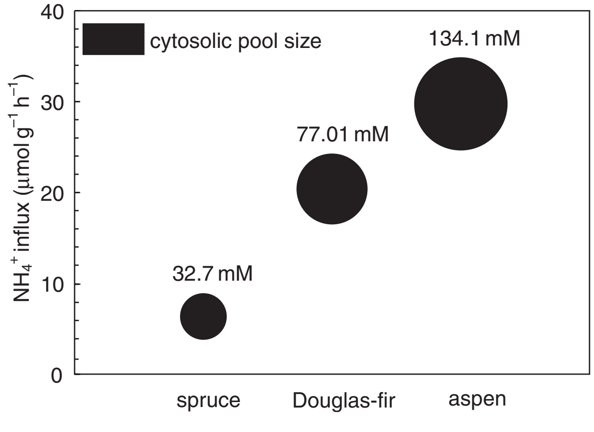

Fig. 4. Pool size of NH_4^+ in the root-cell cytosol, and corresponding root NH_4^+ influx in trembling aspen, Douglas-fir, and white spruce exposed to 1.5 m*M* $[NH_4^+]_o$. Sizes of shaded circles have been calibrated to indicate relative cytosolic concentrations in the three species (absolute values are indicated, in m*M*, above circles). Centres of the circles (*y*-axis values) indicate corresponding unidirectional NH_4^+ influx. Note the significantly larger cytosolic NH_4^+ accumulation in the early-successional species. Data are from Kronzucker et al. (1995a, 1995b), and Min et al. (1999).

Validity of extrapolations to field conditions: N concentrations in forest soils and the role of mycorrhization in high-NH_4^+ soils

Undoubtedly, extrapolation of these findings to successional dynamics is contingent upon a realistic correspondence between laboratory conditions and those in temperate and boreal forest ecosystems. It was particularly important that the external NH_4^+ concentrations used in the studies (0.1 and 1.5 m*M*) reflected both physiological processes and ecological conditions. In the first case, these concentrations are unequivocally representative of nutrient regimes in which the two distinct ranges of NH_4^+ in plant roots, referred to as high- and low-affinity transport, operate (Kronzucker et al. 1996, Min et al. 2000, von Wiren et al. 2000). 0.1 m*M* is frequently used as an N concentration that represents the relatively pure operation of high-affinity systems, while 1.5 m*M* is well into the low-affinity transport range, which involves little or no significant contribution from high-affinity transport. In the second case, these concentrations cover the $[NH_4^+]$ range commonly found in the upper soil horizons (forest floor ‘O’ horizons), the soil layers where seedling establishment takes place (Clements 1928, Etherington 1982, Balisky et al. 1995). High rates of ammonification can produce substantial quantities of NH_4^+ in these layers (Olson and Reiners 1983), and studies examining exchangeable NH_4^+ contents in mature forest floor typically report values that are an order of magnitude higher than corresponding values in mineral soil (van Cleve et al. 1981, Robertson 1982, Vitousek et al. 1982, Olson and Reiners 1983). In a comprehensive study by Vitousek et al. (1982), who present a survey of NH_4^+ contents from 17 forest floor soils, including samples from white spruce, Douglas-fir and aspen forests, realistic solution NH_4^+ concentrations can be derived (assuming, conservatively, that 90% of the KCl-extractable NH_4^+ is bound to cation exchange sites in the soil matrix, and that the water content of the soil is 25%); such solution values fall into a range of approximately 0.30–2.8 m*M*, with a mean concentration of 1.4 mM. Even higher values have occasionally been reported, of up to 15.7 m*M* in brown forest soils in Japan (Ohta and Kumada 1979). More direct soil-water extraction protocols for determining free-solution NH_4^+ have also yielded values up to 2.1 m*M* in late-successional soils (Lodhi 1977, Lodhi and Killingbeck 1980, our unpublished results) and in early-successional soils subject to elevated levels of atmospheric N deposition (van Breemen et al. 1982, Gijsman and de Willigen 1991, Kamminga-van Wijk and Prins 1993). Importantly, such values often represent averages taken over long periods of time, masking seasonal and other transient fluctuations in soil N, which frequently provide NH_4^+ concentrations that are significantly higher (Bormann et al. 1968, Chapin 1980, Binkley and Hart 1989, Rastin et al. 1990, de Visser 1995). In addition, variations in soil water content, which can fall well below the conservative 25% value used above (Gijsman and de Willigen 1991), can further lead to even higher transient and longer-term soil-water NH_4^+ concentrations. Finally, it should be noted that the release of protons that is mechanistically tied to NH_4^+ uptake can decrease rhizospheric pH by as much as 3 pH units relative to the bulk solution, further enhancing, as a result of ion exchange, NH_4^+ availability in the rhizosphere (Runge 1983, Marschner et al. 1991). In summary, the NH_4^+ concentrations used in our study are ecologically relevant, and, appropriately concentrations of 1.5 m*M* and even higher are routinely used in N-transport studies in trees (Chapin et al. 1986, Scheromm and Plassard 1988, Marschner et al. 1991, Flaig and Mohr 1992, Lavoie et al. 1992, Plassard et al. 2000). Finally, it should be pointed out that while NO_3^- and organic N can be present in substantial quantities in many ammonium-rich forest soils (Marschner et al. 1991, Gessler et al. 1998, Nasholm et al. 1998, Rennenberg et al. 1998, Persson and Nasholm 2001), nitrogen uptake by plant roots in high-N-load forest soils is nevertheless almost exclusively in the form of ammonium (Gessler et al. 1998, Rennenberg et al. 1998).

It is also essential to ask whether ectomycorrhizal associations, which are ubiquitously found in roots of these trees, might physically shield the root from high NH_4^+ in the soil solution and thereby attenuate the futile cycling of NH_4^+. Indeed, mycorrhizae can greatly extend the absorptive surface of the symbiotic system and make a significant contribution to phosphate acquisition in trees (Read 1991). However, substantial recent evidence (Eltrop and Marschner 1996, Plassard et al. 2000, Constable et al. 2001) indicates that ectomycorrhizal fungi neither contribute significantly to the acquisition of NH_4^+ by tree seedlings, nor do they prevent NH_4^+ penetration to the surface of the root membrane. A similar condition may also exist with other N sources (Scheromm and Plassard 1988, Persson and Nasholm 2001, cf. Chalot and Brun 1998, Gobert and Plassard 2002). Critically, while mycorrhization may lead to the downregulation of high-affinity influx systems operating at low external NH_4^+, by improving plant nitrogen status through the transfer of organic N from the fungus to the plant (Finlay et al. 1988, Ek et al. 1994), it is well established that high-capacity, low-affinity, influx systems, which operate at moderate to high external NH_4^+, are not subject to downregulation by plant N status and/or by the co-presence of other N sources, but are in fact stimulated by exposure to high NH_4^+ (Wang et al. 1993, Rawat et al. 1999, Min et al. 2000, Cerezo et al. 2001). Importantly, it is these very systems that mediate excessive uptake of NH_4^+ under toxicity conditions. In addition, much evidence shows that the degree of mycorrhization commonly declines with increasing soil N (Menge and Grand 1978, Haug 1990, Taylor and Alexander 1990, Wallander and Nylund 1990, van der Eerden 1998, Britto and Kronzucker 2002), to the extent that it can be used as a sensitive ecological indicator of N deposition (van der Eerden 1998, Wallenda and Kottke 1998). In summary, it is improbable that colonization of tree roots by mycorrhizal fungi can significantly reduce the excessive, and toxic, uptake and futile cycling of NH_4^+. In this light, it is not surprising that toxicity to NH_4^+ is as readily observed in the field as it is in the laboratory (Krajina et al. 1973, Gijsman and de Willigen 1991, Oltshoorn et al. 1991, de Visser and Keltjens 1993, de Graaf et al. 1998).

Conclusions

We argue that the cell-physiological adaptations identified in the present study must have considerable implications for species colonization patterns observed in forest succession. Indeed, it has been demonstrated in herbaceous plants that sensitivity to excess NH_4^+ can lead to species extirpation (de Graaf et al. 1998). Similarly, we believe that the cellular malfunction in NH_4^+ transport efficiency detailed here may, in addition to other factors (see Introduction), provide a physiological basis for the elimination of colonizing tree species such as trembling aspen and Douglas-fir, as forest soils become increasingly depleted in NO_3^- and aggrade towards NH_4^+ domination in the course of ecological succession.

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