Critical ammonium : nitrate uptake ratios for Douglas-fir determining rhizosphere pH and tree mortality

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Abstract

The 'acid rain' problems in forests in The Netherlands are largely due to excessive aerial inputs of NH_3 and NH_4^+ from intensive livestock farms. Nitrification is slow under acid soil conditions, so a large part of the N available to the trees is in the ammonium form. In pot experiments the response of Douglas-fir to various $NH_4^+ : NO_3^-$ ratios was tested, using a nitrification inhibitor. Rhizosphere pH was measured with microelectrodes.

If nitrate contributed less than 20% to total N uptake, net carboxylate production was insufficient for internal pH control and the tree died. If more than 65% of the N absorbed was taken up as nitrate, net OH^- excretion by the roots occurred, leading to alkalization of the rhizosphere at the root tip and at the root growth zone. Thus, root growth can continue in strongly acid soils. At intermediate nitrate contributions to total N uptake, net proton excretion occurred, leading to rhizosphere acidification along almost the entire root. Root growth and functioning can then be impeded due to severe rhizosphere acidity.

Douglas-fir appeared to preferentially absorb ammonium. Soil water content strongly affects the ammonium/nitrate uptake ratio, through its effect on NH_4^+ mobility in the soil.

Introduction

In recent decades, intensification of farming systems has led to an increase in the number of livestock farms, at which enormous amounts of manure are produced. The volatilization of ammonia from the manure gave rise to an increased deposition of NH_x (i.e. $NH_3 + NH_4^+$) onto the surrounding fields and woodlands. It has been postulated that the increased N supply to trees may account for (part of) the forest decline observed recently, due to its effect on soil acidification through nitrification (Van Breemen et al., 1982; 1984) or through proton excretion resulting from ammonium uptake (Van Diest, 1989). Most Dutch woodlands are situated on poor sandy soils, often in areas with heavy concentrations of

intensive livestock farms. It was found that not only was the N availability in the soil relatively high, but also that a strong acidification had occurred, with pH values down to 3.0 in the topsoil.

In 1985 the Dutch Priority Programme on Acidification was initiated in order to unravel the mechanisms involved in forest decline, and to indicate what measures should be taken to stop soil acidification and to preserve the forests for the future. Since N input by deposition was seen as a major detrimental factor, a considerable part of the research was aimed at the direct and indirect effects of N deposition on the ecosystem.

When the NH_x deposited is converted into nitrate by nitrification, the bulk soil will acidify

due to H^+ production, while subsequent uptake of the nitrate formed will result in alkalization of the rhizosphere. If no nitrification occurs, the pH of the bulk soil may rise slightly due to buffering by NH_3, or may drop slightly due to the weakly acidic nature of NH_4^+. Subsequent uptake of the ammonium will lead to acidification of the rhizosphere. Therefore, in studies relating soil acidification to plant growth, it is necessary to know in what form N is absorbed by the plant and to consider the actual rhizosphere pH to which the plant root is exposed rather than the bulk soil pH.

This paper gives a synthesis of the research on the effect of N source on the ionic uptake balance of Douglas-fir (*Pseudotsuga menziesii*) and on the resulting H^+/OH^- excretion and rhizosphere pH.

Materials and methods

All experiments were carried out with 2- or 3-year old Douglas-fir seedlings (*Pseudotsuga menziesii*) [Mirb.] Franco, provenance Arlington 202). The soil used was taken from the research site of the Dutch Priority Programme on Acidification at Kootwijk, The Netherlands. It was a sandy soil of pH-H_2O 3.87 (pH-KCl 3.28), consisting for over 90% of particles $>50\mu$m. The organic matter content was 32 g per kg air-dry soil (loss-on-ignition method). Additional characteristics of the soil were given by Gijsman (1990b) and Tiktak *et al.* (1988).

For explanation of the experimental design of the various experiments, see earlier publications (Gijsman, 1990a; b; c).

Results and discussion

Preference for ammonium or nitrate

The literature on N nutrition of Douglas-fir is contradictory concerning N uptake preference and gr6wth response to either N source. In most experiments on nutrient solution or on sand irrigation culture, ammonium was taken up at a faster rate than nitrate, while growth was also better in the case of ammonium (Littke, 1982; McFee and Stone, 1968; Rygiewicz *et al.*, 1984a; b; Van den Driessche, 1971). In experiments on (non-irrigated) soil, however, nitrate was taken up more rapidly and gave a better growth response (Bigg and Daniel, 1978; Gijsman, 1990b; c; Krajina *et al.*, 1973). It was demonstrated that this apparent contradiction could be accounted for by the difference in mobility of ammonium and nitrate in the soil, due to adsorption of ammonium onto the cation exchange complex (Gijsman, 1990). By comparing the contribution of ammonium to total N uptake with the ammonium /nitrate ratio in the soil solution, it was shown that ammonium was taken up preferentially to nitrate. However, under dry soil conditions ammonium could not reach the roots sufficiently fast, so the preference for ammonium could not express itself. Nitrate being much more mobile, was taken up instead.

Physiological effects of ammonium nutrition

The fact that ammonium is preferentially absorbed by Douglas-fir does not mean that the tree grows best when only ammonium is taken up. It was shown that, when nitrogen was mainly taken up in the ammonium form, a strong reduction in stoichiometric surplus of cation over anion concentration (C-A) in the plant had taken place, resulting in a low carboxylate concentration in the plant (Gijsman, 1990b). Figure 1 shows calculated minimum and maximum net carboxylate production by the plant, in relation to the contribution of nitrate uptake to total N uptake. The net carboxylate production by the plant is drastically reduced when less than 10 to 20% of the nitrogen is absorbed in the nitrate form (*i.e.* NH_4^+/NO_3^- uptake ratio > 4). Since carboxylates are needed for the regulation of the cytoplasmic pH of the plant, a severe reduction can lead to inability to control cytoplasmic pH. With ammonium nutrition under acid soil conditions, there is a heavy demand for carboxylates to eliminate protons from ammonium assimilation and from passive proton influx from the soil solution. A shortage of carboxylates will then result in internal acidification of the plant, and (possibly) high concentrations of NH_4^+-ions in the plant.
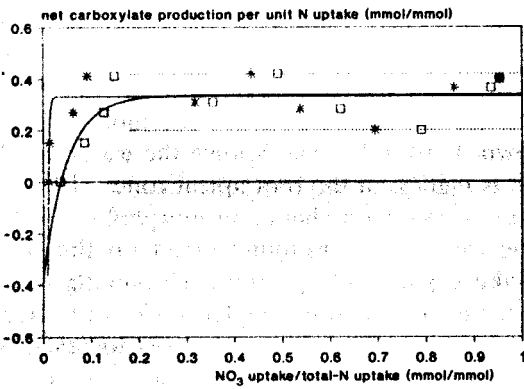
net carboxylate production per unit N uptake (mmol/mmol)

Fig. 1. Calculated net carboxylate production per unit N uptake (mmol mmol^{-1}), as a function of the relative contribution of nitrate to total N uptake. The nitrate contribution could not be determined exactly and is therefore expressed as a maximum (squares) and a minimum (asterisks) value. The solid lines represent the exponential regression lines for the two situations. The broken lines encompass the range of experimental points around the exponentially derived plateau value.

H+ excretion per unit N uptake

Fig. 2. Calculated proton excretion per unit N uptake (mmol mmol^{-1}), as a function of the relative contribution of nitrate to total N uptake. The solid lines represent the proton excretion as calculated from the exponential regression on the carboxylate production in Fig. 1. The broken lines encompass the range of proton excretion, if the measured maximum and minimum values are used in the calculations rather than the exponentially derived plateau value of the carboxylate production.

H^+/OH^- excretion and rhizosphere pH

Besides internal acidification of the plant, the rhizosphere will also become acidified when ammonium is the predominant N source absorbed (Gijsman, 1990c). Using the data of Fig. 1, the proton excretion per unit N absorbed can be calculated, as shown in Fig. 2 (calculation according to Troelstra (1983)). If less than about 65% of the amount of N absorbed consists of nitrate, the roots will excrete protons and the rhizosphere as a whole will thus be acidified. When the nitrate contribution to N uptake is more than about 65% (NH_4^+/NO_3^- uptake ratio < 0.5), OH^- ions are excreted and the rhizosphere as a whole will be alkalized. Differences in excretion pattern among root zones can not be deduced from this figure.

More important than this difference in average rhizosphere pH (pH$_r$) is the difference in the pattern of pH$_r$ along the root axis. Detailed measurements with a microelectrode showed (Gijsman, 1990a) that the rhizosphere was acidified along almost the whole root axis when ammonium nutrition was predominant; only the root tip was slightly alkaline compared with the bulk soil. When more nitrate was absorbed, a stronger alkalization occurred at the root tip and
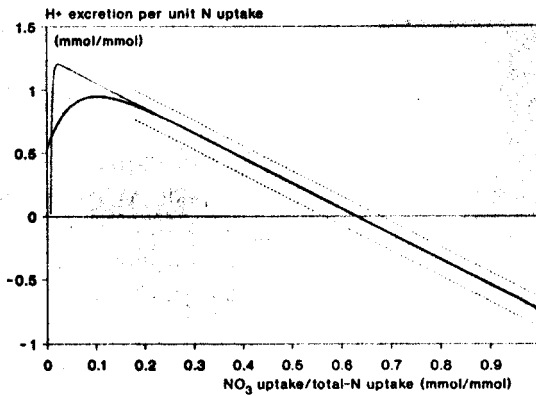
the length of the alkaline zone was much greater. This alkalization could be as high as 0.75 pH unit at the root tip, while the length of the alkaline zone was at most 25 mm. This means that, by absorbing nitrate, the plant is able to increase the rhizosphere pH along its root growth zone, which is the most sensitive part of the root and which is essential for new root formation. The fact that with ammonium–but not with nitrate–root growth was restricted and root tips had a poor, claviform appearance, was possibly connected with the acid conditions in the rhizosphere of ammonium-fed plants.

Consequences of low rhizosphere pH for the roots

In very acid soils, small changes in the rhizosphere pH can determine whether or not a plant can survive. Although no attempt will be made to assess the effects of differences in rhizosphere pH on all factors or processes mentioned, one effect deserves special attention, viz., that of increased aluminium concentration on root growth. Aluminium is often seen as a major detrimental factor in acid soils because of its effect on root cell membranes. Since it was noticed that rhizosphere pH differences were

most pronounced along the root growth zones, the effect of aluminium on root growth and its implications for the plant will be discussed, with emphasis on the water and nutrient uptake capacity of the roots.

Several authors found a negative effect of high aluminium concentrations or high Al/Ca ratios on longitudinal growth of conifer roots (Godbold *et al.*, 1988; Smit *et al.*, 1987). Olsthoorn (1990) found that the specific root length of Douglas-fir (*i.e.* root length per unit root dry weight) was negatively correlated with the aluminium concentration in the soil solution. The much lower rhizosphere pH along the root zones of ammonium-fed plants compared with nitrate-fed plants will lead to higher aluminium concentrations in the soil solution, which may result in thicker and–assuming a constant carbohydrate investment in the roots–shorter roots. Both phenomena were indeed observed in plants with different N nutrition.

A reduction in root length may have important consequences for the nutrient uptake capacity of the roots, especially for less mobile nutrients as phosphorus. Since the transport rate of these nutrients towards the roots is low, a high root length density is needed to create a low nutrient demand per unit root length and to reduce the transport distance of the nutient towards the roots (Van Noordwijk and De Willigen, 1979; De Willigen and Van Noordwijk, 1987). Also mycorrhizae, with their fine network of fungal hyphae, may increase soil penetration, contributing to nutrient uptake. However, it has been found that several mycorrhizal fungi of Douglas-fir are very sensitive to low pH and to a high aluminium concentration in the rooting medium (Jongbloed and Borst Pauwels, 1987; Kamminga-Van Wijk and Prins, 1988). The uptake capacity of the roots for nutrients of low mobility may thus be reduced by increased acidity in the rhizosphere.

In many Dutch coniferous forests, water availability is a critical factor for the trees, so changes in rooting pattern or in root functioning may be of vital importance. The water uptake capacity of the roots depends on several morphological and physiological root parameters, viz., root length, root resistance to water entry and longitudinal hydraulic conductivity of the root xylem.

Root length density in the soil is the decisive factor in water uptake capacity of the roots, insofar as water availability is restricted by water transport limitations in the soil (De Willigen and Van Noordwijk, 1987). Furthermore, it was shown that for Norway Spruce the water uptake rate is highest in the root apical zone (Häussling *et al.*, 1988), so a change in morphology of this zone can have a profound effect on the water uptake capacity of the root. Thickening of the roots means that water molecules have to travel a greater distance within the roots to reach the root xylem, from where they are transported upwards to the shoot. This will cause a greater root resistance to water entry. Effects of aluminium on entry resistance of the root xylem were only recently demonstrated by Kruger and Sucoff (1989). They found that prolonged exposure of *Quercus rubra* roots to high Al/Ca ratios resulted in increased entry resistance of its roots. It can thus be concluded that a lower rhizosphere pH along the root growth zone of ammonium-fed plants can reduce the water uptake capacity of the root through changes in root morphology.

Synthesis and conclusions

The present investigation was carried out with 2- or 3-year old Douglas-fir seedlings, under (semi-) controlled experimental conditions. Older trees may show a different physiological response to external conditions and, moreover, will often have a higher 'buffer capacity' for temporarily unfavourable conditions. For instance, older trees are likely to have a much higher carboxylate content than young trees, and thus will be able to longer endure a situation with a high carboxylate demand but a low carboxylate production. Under forest conditions the root system is much more extensive than in the experimental pots, so that the plant can avoid spots with adverse soil conditions by reducing root growth in one place and increasing it in another. Carboxylates originating from nitrate-absorbing roots may be decarboxylated by ammonium-absorbing roots, which leads to OH^- excretion and thus a smaller pH reduction along ammonium-absorbing roots. The results therefore have to be interpreted with some caution for trees in a forest situation.

The effect of N source on carboxylate concentrations and on the rhizosphere pH can be summarized for three situations shown in Fig. 3 (in reality the boundaries between zones will not be as sharp as suggested here).

1. If more than about 65% of the N absorbed is taken up as nitrate, the carboxylate concentration in the plant is at a proper level and the roots excrete OH⁻ ions. This is a favourable situation for plants in acid soils, as the rhizosphere is alkalized, especially along the root growth zones.

2. If nitrate contributes about 20 to 65% of total N uptake, the net carboxylate production by the plant is still high enough, but with decreasing nitrate contribution the roots excrete increasing amounts of H$^+$ ions. This will lead to acidification of the rhizosphere along most parts of the roots. Only at the root tip may a slight alkalization remain.

3. If less than about 20% of the amount of N absorbed consists of nitrate, the carboxylate production in the plant decreases and physiological disorders may occur. The roots excrete H$^+$ ions and thus acidify the rhizosphere. Judging from the poor appearance of the root tips, root growth and functioning are probably disturbed.

It may be concluded that Douglas-fir, although it preferentially absorbs ammonium compared to nitrate, requires part of its N in the form of nitrate. Since deposition occurs in ammonium (or ammonia) form, the source of N available to the plant depends on the rate of nitrification. When little nitrification occurs, nitrogen is mainly available to the roots in the ammonium form. This situation is detrimental for the plant. As long as part of the NH$_x$ is nitrified, the roots have an opportunity to absorb some nitrate as well. Nitrification, therefore, is a crucial factor in the prospects for survival of Douglas-fir in situations with high NH$_x$ input.

Even if both N sources are present in the soil, it is still questionable whether or not the roots will absorb some nitrate. In the case of an absolute preference for ammonium compared to nitrate, no nitrate will be taken up at all if sufficient ammonium is available to meet total N demand. The plant will then, through its ammonium preference, create its own detrimental situation. Experimental results from Keltjens and Van Loenen (1989) and Gijsman (1991), however, suggest that there is no absolute ammonium preference, so there might be some kind of self-protecting mechanism ensuring that the plant absorbs at least some nitrate, if available.

Given a certain concentration of ammonium and nitrate in the bulk soil, their availability at the root surface is controlled by soil water conditions and ammonium adsorption onto the exchange complex. Under dry soil conditions the mobility of ammonium may be so low that it cannot reach the roots in sufficient amounts to meet the plant's N demand. Some nitrate will then be taken up also. A wetter soil will increase ammonium mobility, leading to a reduced contribution of nitrate to total N uptake. Since in a forest most of the ammonium will be concentrated in the dry upper soil layer, a flush of ammonium uptake may be expected after a shower. From the point of view of N nutrition, relatively dry soil conditions may therefore be favourable for the plant when NH$_x$ input is high. However, this conflicts with the fact mentioned earlier that, in the forest soils considered, water availability is often critically low.

For good growth and functioning of Douglas-fir forests a substantial reduction of NH$_x$ input is required, to a level where most of the N deposited can be nitrified, so that the larger part of the N taken up by the plant consists of nitrate.
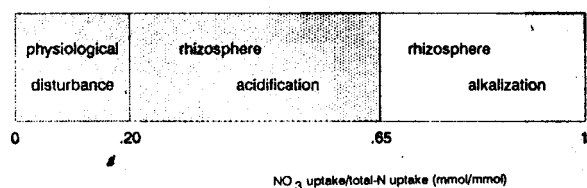

Fig. 3. Schematic representation of the conclusions of the present research on the response of Douglas-fir to ammonium and nitrate nutrition. Numbers represent the relative contribution of nitrate uptake to total N uptake.

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