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Influence of ammonium on fine root development and rhizosphere pH of Douglas-fir seedlings in sand

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Abstract

Ammonium sulphate is a major component of the air pollutants deposited on forests in the Netherlands. Different amounts of NH_4^+ were added to Douglas-fir seedlings grown in tall containers of sand, to study the influence of high concentrations of NH_4^+ in the soil on the development of fine roots and the effects of nitrogen uptake on rhizosphere pH. At the end of this eight-month experiment part of the ammonium appeared to have nitrified into nitrate. High doses of ammonium negatively affected root length and root length per unit of dry matter (specific root length). Although Douglas fir shows a preferential ammonium uptake in nutrient solutions the increases in the pH of the rhizosphere in this experiment indicate that nitrogen was mostly taken up as nitrate. When the ammonium concentration in the soil is low, it cannot be taken up readily because of its low mobility in soil. Shoot growth was stimulated by high availability of nitrogen. The possible effects of high doses of ammonium on long-term forest vitality are discussed.

Introduction

Ammonium originating from manure produced by intensive livestock farming is a major component of the total deposition of air pollutants in the Netherlands, where the annual deposition of NH_4^+ can exceed 100 kg N per hectare. When ammonium (NH_4^+) is converted into nitrate (NO_3^-) by nitrification, the soil acidifies (Van Breemen *et al.*, 1982). The acidification of forest soils causes cations (K, Ca and Mg) to leach out and aluminium to go into solution (Mulder *et al.*, 1987). Large amounts of aluminium ions in the soil solution, particularly in combination with small concentrations of Ca and Mg, have been shown to be toxic to several forest tree species (Eldhuset *et al.*, 1987).

Ammonium can have a fertilizing effect on forest stands when N availability limits growth. However, excess nitrogen influx to forests on infertile sand soils causes an imbalance in nutrient supply to trees and may induce deficiencies of other nutrients such as P, K and Mg. Moreover, the NH_4^+ may accumulate in the rooting medium, reaching toxic levels and impairing root development and plant functioning (Keltjens and Van Ulden, 1987).

Since plants require much more nitrogen than others nutrients, the form (NH_4^+ or NO_3^-) in which nitrogen is taken up by the roots largely

determines the ionic uptake balance and rhizosphere pH. Uptake of N in the form of NH_4^+ results in a cation/anion uptake ratio >1 and electrical neutrality will thus be maintained by a net efflux of protons from the roots. This will cause rhizosphere pH to fall. The uptake of NO_3^- mostly results in a cation/anion ratio <1, with a corresponding increase in the rhizosphere pH (Gijsman, 1990; Rygiewycz *et al.*, 1984a, b). As the Al activity increase with decreasing pH, NH_4 uptake may result in more pronounced effects of Al toxicity.

In our experiment we added NH_4 directly to the soil and examined the effects on the root development and rhizosphere pH of Douglas-fir seedlings in a greenhouse. We assessed the chemical composition of the soil and shoot and root parameters of the Douglas-fir seedlings at the end of an eight-month growing period.

Materials and methods

In May 1986 one-year-old nursery grown Douglas-fir seedlings (*Pseudotsuga meziesii* (Mirb.) Franco; provenance Arlington 202) were planted in 25-litre stainless steel containers. The containers were filled with inland dune sand (CEC around 2 mmol (+) kg^{-1} soil; organic matter 0.5%; pH–H_2O 4.7), covered with 2 cm of fine gravel to reduce evaporation. A single plant was grown in each container and there were four containers per treatment. All seedings were supplied with nutrients sufficient for a dry matter production of 10 g per plant, calculated according to Fiedler *et al.* (1985) and Larsen (1976), and including 1.5 mmol NH_4^+ per plant. The 4 treatments were as follows (the treatment code in brackets refers to the corresponding nitrogen deposition, in kg N per hectare): (N0) no NH_4 added, (N17) 6 mmol NH_4/plant, (N140) 48 mmol NH_4/plant, (N340) 120 mmol NH_4/plant. The NH_4 was supplied as ammonium sulphate in five equal portions at weekly intervals during July and August. The moisture content in all containers was monitored with small tensiometers and kept at pF 1.6. When necessary, demineralised water was added to compensate for evapotranspiration. No water leaked from the containers, although measurements at the end of the experiment showed that the moisture contents were slightly higher at the bottom. From September onwards artificial lighting was used to maintain day length at 14 hours. Greenhouse temperature was kept at 20°C.

All plants were harvested in January 1987. The dry weights of the woody parts of the shoots and of the needles were measured. The pots were then frozen for 14 h at −20°C, and the resulting solid soil/root mass was tipped out and sawn into four layers 14, 10, 10 and 14 cm thick. Per layer, the soil was sampled for chemical analysis and estimation of the moisture content. The roots from each soil layer were collected directly after thawing, to ensure that the uptake behaviour was still reflected in the rhizosphere pH. The roots were dried at room temperature for 30 minutes and then the rhizosphere soil was collected by shaking the roots over a tray. All roots were then washed over a sieve and their length and dry weight were recorded. The pH of the bulk soil was measured in a mixed soil sample from each layer. Rhizosphere pH and bulk soil pH were both measured using a 1:5 ratio of air-dry soil to water. Total root length was estimated by the line intersect method (Newman, 1966; Tennant, 1975). Plant dry weight was measured after drying at 70°C for 16 hours. Moist soil samples were extracted with 1 *M* KCl to find the NO_3, NH_4, Al, Ca and Mg contents. Chemical analyses were performed as described by Keltjens and Van Ulden (1987).

Results

Soil chemistry

In the control and lowest ammonium treatments (N0 and N17) almost no ammonium was present in the soil at the end of the experiment (Fig. 1a), but nitrate was present in considerable amounts (Fig. 1b). In the N140 and N340 treatments more NH_4 and NO_3 had accumulated in the soil. In treatment N340 the NH_4 concentrations in the soil were much higher than in treatment N140, but the concentrations of NO_3 were the same in both treatments. At all four levels of NH_4 application the more mobile NO_3 was found mainly in the lower part of the containers, where it had

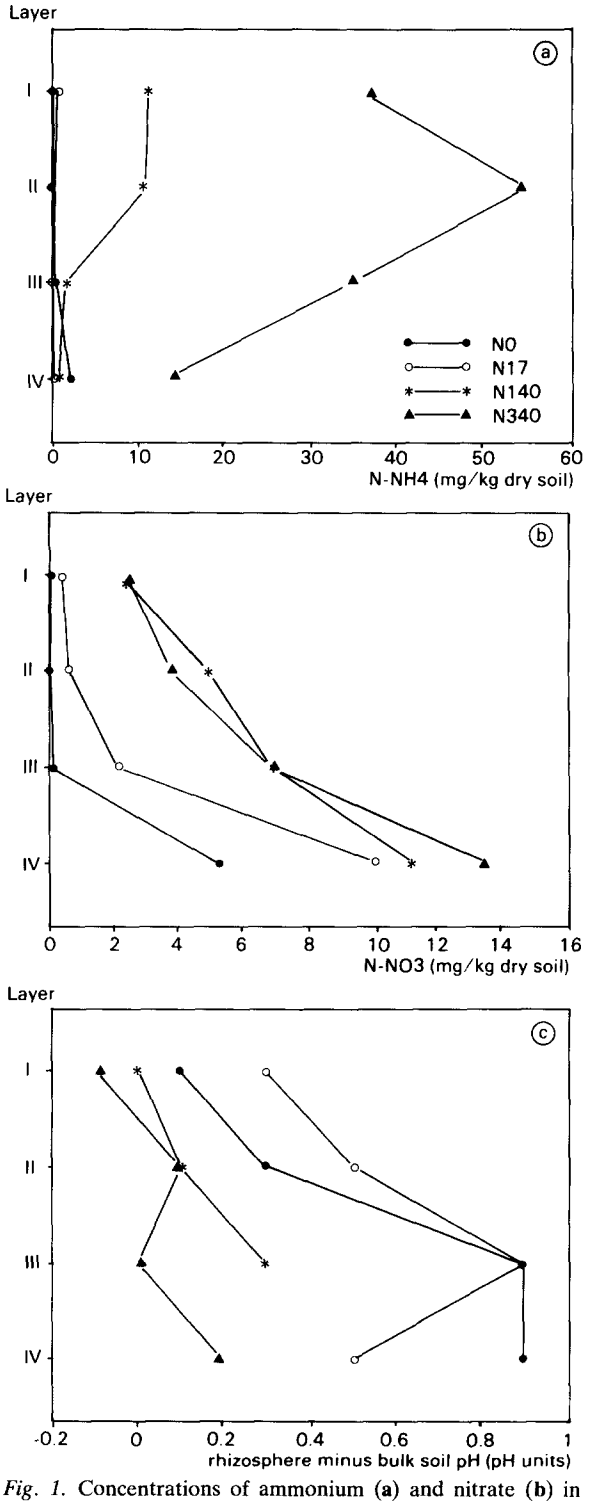

Fig. 1. Concentrations of ammonium (**a**) and nitrate (**b**) in four layers in containers (50 cm tall) of dune sand. Difference between rhizosphere pH and bulk soil pH in the same layers (**c**). Each point is an average of four containers. Measurement at the end of an eight-month growing period.

moved with the net downward movement of water.

The pattern of NH_4 and NO_3 concentrations in the soil in the four treatments suggests that the soil has a maximum nitrification capacity. At low levels of NH_4 supply (N0 and N17) all NH_4 is nitrified or taken up, but the NO_3 accumulates. Maximum nitrification capacity is attained at a level between N17 and N140. At higher NH_4 supply, nitrate concentrations in the soil remain constant, while NH_4 concentrations increase.

In almost all rhizosphere soil samples the pH was higher than that in the bulk soil, even at high levels of NH_4 supply (Fig. 1c; Table 1). In treatments N0 and N17, the pH increase in the root zone was large, especially near the bottom of the containers, where ample NO_3 and little NH_4 was present. The rise in rhizosphere pH was smallest in the uppermost layer of treatments N140 and N340, where large quantities of NH_4 were present.

No significant differences between the four treatments developed in the pH of the bulk soil (Table 1), or between layers within the containers. At high NH_4 supply more Al is adsorbed and adsorbed Ca tends to decrease (Table 1); therefore the Al/Ca ratio in the soil increases with increasing NH_4 supply. In the two highest NH_4 treatments this ratio reached such high levels that damage to the fine roots can be expected (Meiwes *et al.*, 1986). The NH_4/Ca ratio was especially high in treatment N340 (Table 1).

Plant growth

Total root length and root distribution in treatments N0, N17 and N140 was similar (Table 2; Fig. 2a). In treatment N340 root length was depressed, especially in layers I and II, where levels of NH_4 were high (see also Fig. 1a). The specific root length (SRL; ratio of root length to root dry weight) decreased from 14.1 m/g dry matter in treatment N0 to 8.3 m/g dry matter in treatment N340, mainly because of reduced root length, whereas root dry weight was decreased only slightly by high additions of NH_4 (N340). Figure 2b shows the SRL in the different layers of the treatments. The lower SRL in layer I of each treatment is a result of the higher number

Table 1. Chemical parameters of the soil, supplied with four different levels of ammonium sulphate. Data are averaged over four layers per pot, and determined at the end of the experiment. Treatment names refer to the equivalent nitrogen supply in kg N per hectare. (n = 4; standard deviations in brackets)

Treatment	N0	N17	N140	N340
NH_4^+ added: (mmol/container)	1.5	7.5	49.5	121.5
Soil parameters				
Bulk pH–H_2O	4.1 (0.1)	4.0 (0.0)	3.9 (0.1)	4.0 (0.1)
Rhizosphere pH–H_2O	4.7 (0.4)	4.6 (0.3)	4.1 (0.2)	4.1 (0.1)
Rhiz. pH minus bulk pH	0.6 (0.4)	0.6 (0.3)	0.2 (0.2)	0.1 (0.2)
NO_3^- (mg N kg^{-1} dry soil)	1.6 (1.2)	3.6 (0.7)	6.4 (1.1)	6.9 (1.6)
NH_4^+ (mg N kg^{-1} dry soil)	0.7 (1.1)	0.2 (0.4)	6.3 (1.3)	33.4 (4.3)
Al^{3+} (mg kg^{-1} dry soil)	25.0 (1.3)	27.9 (0.8)	33.4 (1.3)	33.1 (2.5)
Ca^{2+} (mg kg^{-1} dry soil)	14.9 (8.6)	12.5 (5.6)	11.1 (3.7)	11.1 (10.6)
Al^{3+}/Ca^{2+} ratio (mol/mol)	2.4	3.3	5.0	5.0
NH_4^+/Ca^{2+} ratio (mol/mol)	0.13	0.05	1.62	8.61

Table 2. Root and shoot characteristics of Douglas-fir seedlings grown for 8 months in sand with different additions of ammonium sulphate. For description of treatments see Table 1. (n = 4; standard deviations in brackets)

Treatment	N0	N17	N140	N340
Plant parameters				
Total root length (m/plant)	40.7 (13.7)	37.6 (16.1)	35.4 (7.3)	20.4 (2.8)
Root dry weight (g/plant)	3.3 (0.8)	3.9 (1.1)	3.4 (0.8)	2.9 (0.8)
SRL[a] (m/g d.m.)	14.1 (2.4)	10.4 (2.1)	12.1 (2.6)	8.3 (1.0)
Shoot dry weight (g/plant)	2.7 (0.8)	3.4 (0.7)	3.5 (1.5)	3.7 (1.2)
Shoot/root ratio	0.8 (0.1)	0.9 (0.2)	1.0 (0.2)	1.3 (0.1)

[a] Specific root length of roots <2 mm diameter.

of structural roots in the fraction of fine roots (<2 mm diameter). In layer IV, the lower SRL might be the result of slight soil compaction. The specific root length was smallest in the layers with the highest concentrations of NH_4.

Higher shoot dry weights at higher nitrogen levels (Table 2), point to the fertilizing effect of NH_4 in the experiment. The stimulated shoot growth caused the shoot/root ratio in the highest treatment (N340) to be nearly double that of the control plants (N0).

Discussion

Nitrification and soil chemistry

In treatments N0 and N17, almost all NH_4 was nitrified or taken up during the experimental period, resulting in NO_3 being the main N source at the end of the experiment. The maximum nitrification rate was found in treatments N140 and N340. Here there was a mixture of NH_4 and NO_3 in the soil at the end of the experiment.

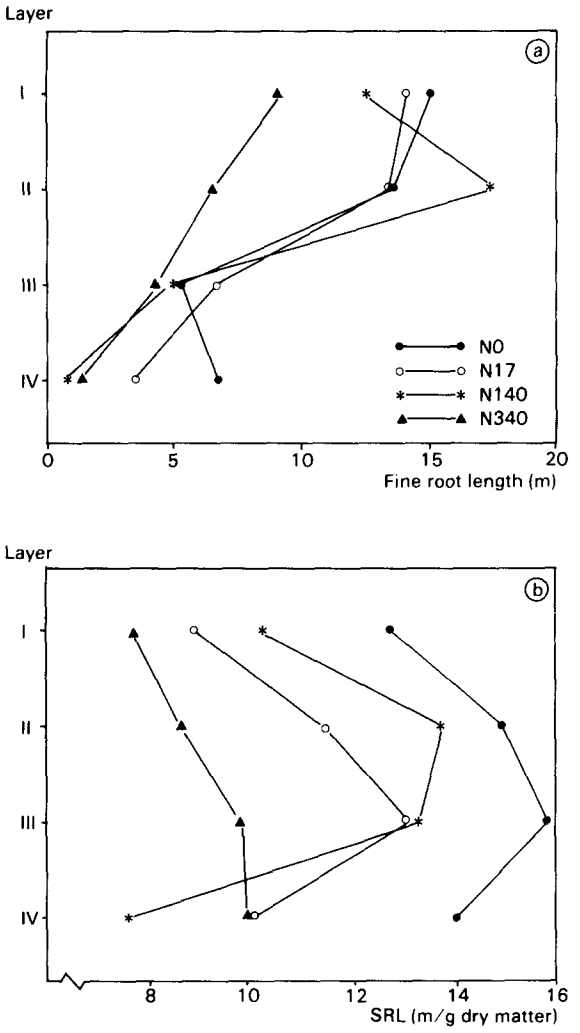

Fig. 2. Total root length (**a**) and specific root length (SRL) (**b**) of 1-year-old Douglas-fir seedlings, in four layers in containers. Each point is an average of four containers. Measurements at the end of an eight-month growing period.

Figures 1a and 1b illustrate the differences in mobility between NH_4 and NO_3 in the soil. Although all NH_4 was applied at the start of the experiment, at the end of the experiment most was still found in the two top layers. The net water movement in the soil was downward, although no water drained from the container. This was reflected by the high concentrations of NO_3 in the deeper soil layers.

Initially the pH of the soil was 4.7, but by the end of the experiment it was around 4.0 in all treatments. During nitrification H^+ ions are produced and therefore the pH in all treatments should have fallen considerably. In treatments N140 and N340 more H^+ was produced than necessary for this drop in pH; this was found from a titration curve made for this soil. The surplus H^+ generated in treatments N140 and N340 was buffered by Al going into solution. In these cases, the Al concentration was indeed significantly higher than in the two lowest treatments (Table 1). Calcium concentrations in the soil declined as NH_4 supply increased. As a result the Al/Ca ratios in treatments N140 and N340 reached levels at which root damage may occur. According to Meiwes *et al.* (1986) a molar Al/Ca ratio of >5 in a solution culture can damage fine roots of Norway spruce.

Nitrogen uptake

In solution culture, Douglas fir shows a preference for NH_4^+ rather than NO_3^-. The results obtained by Keltjens and Van Loenen (1989) show that in an equimolar NH_4NO_3 solution, 65% of the nitrogen is taken up as NH_4 (at pH 3.8). In our experiment, the pH of the rhizosphere was mostly higher than that of the bulk soil. This indicates that uptake of NO_3 was dominant. Nitrification and the differing mobility in soil resulted in different ratios between NO_3 and NH_4 in each treatment, as well as between the soil layers in the containers. In treatments N0 and N17 the main nitrogen source was NO_3. This is why the pH in the rhizosphere was higher. Moreover, in treatments N140 and N340 the rhizosphere pH was often higher than the bulk soil pH, despite the presence of a mixture of NH_4 and NO_3. This implies that the supply of NH_4 to the roots was limited by the lower mobility of NH_4. Where NH_4 is present in large concentrations its transport to the root surface is not limited. NH_4 is then taken up in sufficient quantities to keep the rhizosphere pH close to the pH of the bulk soil (Figs 1a and 1c). In all treatments the difference between rhizosphere pH and bulk soil pH was greater in the deeper layers of the container, where NH_4 was in short supply.

Effects on root growth

Root development was severely inhibited at the

highest ammonium supply. In treatment N340 the Al/Ca ratio in the soil was the same as in treatment N140, but the NH_4/Ca ratio was much higher. At high NH_4 levels (layers I, II and III in treatment N340) the specific root length was very low compared with the control (N0). Van den Driessche (1978) also found that when growing Douglas-fir seedlings at pH 4 in a sand culture the SRL was higher with NO_3^- than with NH_4 nutrition. However, the reduced root development we found at the highest treatment cannot be attributed to NH_4 only, because the direct effects of NH_4 are confounded by the influence of NH_4 on the rhizosphere pH and the Al activity in the rhizosphere. These primary and secondary effects of NH_4 on root growth cannot be distinguished in our experiment.

Given the preferential uptake of NH_4 and its effect on rhizosphere pH, some conclusions can be drawn about rhizosphere changes during root growth in a soil containing both NO_3 and NH_4. When a root tip enters a volume of fresh soil it will take up the available NH_4. As a result, the rhizosphere pH will decrease, with negative effects on growth, as described above. This implies that root growth is reduced until the NH_4 in the rhizosphere is depleted, and rhizosphere pH increases because of uptake of NO_3. If this process continued, the net result would be a severely restricted root growth in soil, having a low pH and much available NH_4. Recently, Gijsman (1990) found that the pH of the rhizosphere at the root tip of Douglas fir is usually higher than that of the rhizosphere further back. If uptake behaviour is different at the root tip, this may favour root growth under these conditions. However, if NH_4 uptake starts in the zone of root elongation this may still imply that root tip growth is slowed down by high NH_4 levels in acid soils. Pursuing this argument, Gijsman (1990) concludes that the presence of NO_3 is essential for the normal functioning of Douglas-fir roots in acid soils.

Long-term effects of ammonium deposition on forest vitality

High rates of ammonium deposition lead to acidification of forest soils (Van Breemen *et al.*, 1982) and may result in nutrient imbalances of forest stands (Van den Burg and Kiewiet, 1989). Excessive N nutrition may make trees more susceptible to disease (De Kam *et al.*, 1989) and to frost (Aronsson, 1980). Our experiment shows that high levels of NH_4 deposition may have strong negative effects on root growth under field conditions: root densities will decrease and root uptake capacity will be reduced. At the same time, shoot growth may be stimulated by increased N availability, especially on formerly N-deficient sites. The transpiration of the forest stand may increase, and on dry soils this will lead to larger water deficits.

Linder *et al.* (1987) reported the effects of drought on the survival of *Pinus radiata* after heavy fertilization in the Australian 'Biology of Forest Growth' project. Although they did not present data on root growth it is clear that the shift in shoot/root ratio caused by fertilization (see also Linder & Axelsson, 1982) decreased the chances of survival. In their control treatment no trees died from drought, although there was severe needle loss. But in the fertilized stand 7% of the trees died. Drought periods in the Netherlands might not be as severe as in Australia, but this example shows that the risks of tree mortality may increase under Dutch levels of atmospheric deposition of pollutants, especially nitrogen compounds.

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