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The effect of air-borne ammonium sulphate on *Pinus nigra* var. *maritima* in the Netherlands

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Summary As a result of air pollution, considerable deposition of ammonium sulphate occurs on vegetation and soil in the vicinity of chicken farms and fields dressed with animal slurry. A clear relation exists between this ammonium sulphate deposition and the distance to certain agricultural activities. Field investigations and ecophysiological experiments both show that the needles of *Pinus nigra* var. *maritima* (Ait.) Melville take up ammonium and excrete potassium, magnesium and calcium. This often results in potassium and/or magnesium deficiencies and may lead to premature shedding of needles. The high levels of nitrogen in the needles are strongly correlated to fungal diseases.

Whether the osberved cation leaching will result in disturbed nutrient budgets depends mainly on soil conditions. Leaching of K, Mg and Ca from the soil, caused by ammonium sulphate, may further inhibit nutrient uptake.

Field investigations show a clear correlation between increased ratios of NH_4 to K, Mg and Ca in the soil solution and the damage to pine forests.

Introduction

Recent publications concerning forest damage in Western-Europe have expressed considerable alarm, particularly in Germany where the vitality of the forests has decreased rapidly. Air pollution is generally believed to be the main cause. Ulrich[20] ascribes the phenomena mainly to soil acidification as a result of acid or acidifying substances from the atmosphere. The high Al/Ca ratio resulting from dissolution of aluminium by mineral acids and from leaching of calcium is harmful to the root system. Krause *et al.*[11], however, believe that the observed nutrient deficiencies and concomitant damage to the trees can be ascribed mainly to leaching as a result of ozone damage to the leaves. In the Netherlands the condition of pine forests is often poor. In 1983 a nation-wide investigation, carried out by the Dutch State Forest Service (den Boer and Bastiaens[2]), showed that 7 to 38% of all trees in *Pinus sylvestris* L. forests had less than one complete year-class of needles. In *Pseudotsuga menziesii* (Mirb.) Franco forests, 29 to 70% of the trees had less than 50% of the leaves considered normal for this climatic zone. 64% of the unhealthy *Pinus sylvestris* forests and

41% of the unhealthy *Pseudotsuga menziesii* forests showed grey-green or yellow-green discoloured needles.

Janssen[8] and Hunger[6] noticed a relation between intensive stock-breeding and the condition of pine trees.

On an average the total deposition of acid and potential acidifying substances in The Netherlands is ± 6 kmol equivalent $H^+ \cdot ha^{-1} \cdot yr^{-1}$ of which ± 80% as $(NH_4)_2SO_4$ [1].

Van Breemen *et al.*[3,4] state that ammonia from manure reacts with sulphur dioxide from the atmosphere, causing wet and dry deposition of large amounts of ammonium sulphate on trees and soil[19]. In a woodland near Warnsveld nitrification of the ammonium caused a marked pH decrease of the soil (temporarily down to pH 2.8).

In the present study precipitation analyses have been carried out at various distances from chicken farms and fields dressed with animal slurry. The chemical compositions of open field precipitation and forest canopy throughfall have been compared.

In the South-east of the Netherlands soil and needle analyses have been carried out on *Pinus nigra* var. *maritima* (Ait.) Melville at 58 locations and the results were correlated with the condition of the trees. Acid and artificially buffered soils were incubated after adding various amounts of ammonium sulphate to investigate nitrification processes. Eco-physiological experiments were carried out in order to estimate the leaching of nutrients from needles caused by ammonium sulphate.

Materials and methods

Field investigations and experimental studies

During 1983, fortnightly precipitation was collected and analysed, using black polyethylene bottles (1 l) and funnels (ϕ 10 cm), containing 1 ml of a 200 mg.l^{-1} $HgCl_2$ fixing solution. In the spring and autumn 58 locations with *Pinus nigra* var. *maritima* were visited. At each location soil samples were taken, consisting of six subsamples of the mineral sandy soil, taken just below the litter layer to a depth of 15 cm, using a brass tube (length 15 cm, ϕ 18 cm). The samples were transported to the laboratory in a refrigerated container.

Needles were collected from each year class from trees between 30 and 50 years old. The vitality was determined from the number of year classes of needles. Trees with 2–3 year classes were estimated as healthy, those with 1–2 year classes as moderately damaged and those with 0–1 year class as severely damaged. In order to analyse an aqueous soil extract 70 g of fresh, well mixed soil were weighed into a 500 ml polyethylene bottle together with 200 ml of twice-distilled water and shaken on a Gerhardt model LS 20 shaker for 1 hour. The pH was then measured with a Metrohm model E 488 pH/mV meter and a model EA 152 combined electrode. After centrifugation in a Heraeus Christ model 3 labofuge (10 min, 5000 rpm) 100 ml of the supernatant were fixed with 0.5 ml of 200 mg.l^{-1} $HgCl_2$ solution and stored at – 28°C until analysis. For the tissue analyses 50 mg of dried (72 h 55°C) and ground needles were digested according to Nkonge[13].

Nitrification and soil acidification experiments were conducted with a mixture of ten acidic forest soils (pH H_2O 4.1). A portion of the mixture was artificially buffered with

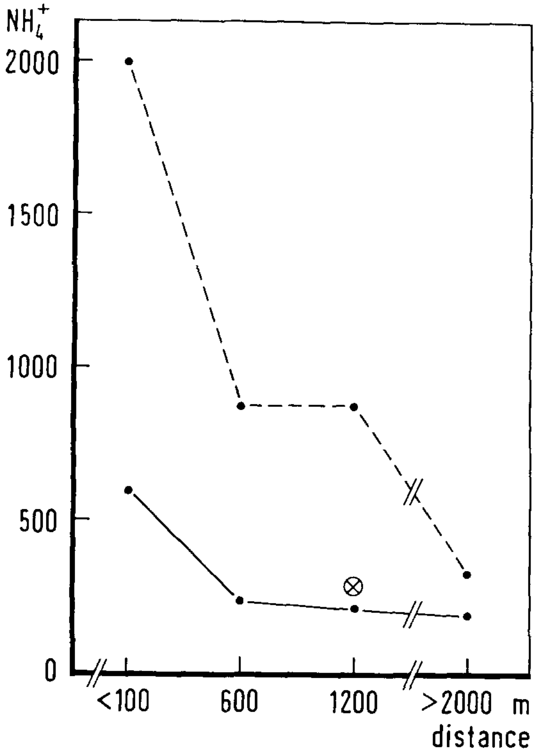

Fig. 1. The ammonium concentration in —— open field precipitation (μM) and ----- forest soil (μmol kg^{-1} dry wt) as a function of the distance to chicken farms.

Table 1. pH and chemical composition (averages) of soil distilled water extract (1:3) in **A**: healthy, **B**: moderately and **C**: severely damaged *Pinus nigra* var. *maritima* forests (μmol.kg^{-1} dry soil)

	n	pH (H_2O) Mean	Min	Max	NH_4	NH_4(KCl)	NO_3	K	Mg	Ca	Al
A	20	4.1	3.5	4.6	334	687	271	137	77	153	191
B	16	4.0	3.4	4.9	384	751	130	47	45	128	158
C	20	4.1	3.7	4.4	509	1346	117	60	26	43	183

50 mmol $CaCO_3$. kg^{-1} (pH H_2O 6). To 600 g portions of the artificially buffered fresh soil (moisture content 21%), 0, 5, 10, 15, 20, 25 and 38 mmol of $(NH_4)_2SO_4$ were added per kg of fresh soil; the untreated mixture received 25 mmol . kg^{-1}. All soils were incubated at 20°C for 114 days, covered with glasswool to prevent evaporation. The pH was measured fortnightly and the chemical composition was estimated at the beginning and end of the 114 days period.

Nutrient uptake/release experiments were carried out with $\frac{1}{2}$ year old needles of a healthy *Pinus nigra* var. *maritima* tree from a forest near Nijmegen. After collection, the needles were washed thoroughly with twice distilled water. Five 50 g (wet weight) portions of needles were incubated (24 h, 20°C) in duplo in 2 l perspex containers, containing 0.5 l bidistilled water and 1: 0 μM $(NH_4)_2SO_4$, 2: 250 μM $(NH_4)_2SO_4$, 3: 250 μM Na_2SO_4, 4: 2500 μM $(NH_4)_2SO_4$ and 5: 2500 μM Na_2SO_4. The containers were placed on a Gerhardt model 20 shaker and irradiated with a Philips type HP (1) 400 W high pressure metal halide lamp at a light intensity of 500 $\mu E \cdot m^{-2} \cdot s^{-1}$. During the experiment the pH in all media was 4.35 ± 0.15. 10 ml water samples were taken at 0, 1, 4, 8 and 24 h after the start of the experiment and analysed immediately after completion of the experiment.

Calcium and magnesium contents were estimated with a Beckman model 1272 A.A.S., and aluminium with a LI type V11 flameless A.A.S. Potassium was estimated flame-photometrically using a Technicon I Auto-Analyzer. A Technicon II Auto-Analyzer was used for the colorimetric analyses of nitrate according to Kamphake *et al.*[9], ammonia by the method of Grasshof and Johannsen[5], sulphate by the Technicon methodology[18] and chloride according to O'Brien[15]. Statistical analyses of the results were carried out according to Kruskal-Wallis.

Results

Effects on soil

The ammonium levels of the forest soils and the ammonium sulphate levels in the precipitation increase strongly with decreasing distance from chicken farms (Fig. 1). In all forest soils investigated, however, nitrate levels were low and ammonium appeared to be the preponderant nitrogen source.

The average pH (H_2O) was the same for healthy, moderately damaged and severely damaged forests (pH 4.1, Table 1). The results from the incubation experiments demonstrated that nitrification proceeded rapidly in artificially buffered soils leading to a rapid decline in pH (Fig. 2). With sufficient ammonium sulphate present the pH decreased to 4.1. The pH did not decrease further in the presence of ammonium sulphate even after 114 days (Fig. 3).

The NH_4 levels (Table 1) in soil solutions of severely damaged forests are significantly higher than in healthy forests (P = 0.045), whereas the Mg and Ca concentrations were significantly lower (P = 0.008 and 0.004). Also the K concentrations seem to be lower in the soils of severely damaged forests but these differences are not significant (P = 0.08). The aluminium concentrations were almost equal for healthy, moderately damaged and severely damaged forests. As a result the NH_4/K, NH_4/Mg and Al/Ca ratio are relatively low in healthy forests and significantly higher in severely damaged forests (P = 0.010, P = 0.003 and P = 0.017). (Table 2).

Effects on leaves

Typically characteristic of damaged trees are premature shedding of needles and the frequent occurrence of fungal diseases. Tissue analyses show that there are only slight differences between the potassium and magnesium levels in one, two and three year old needles of non-damaged

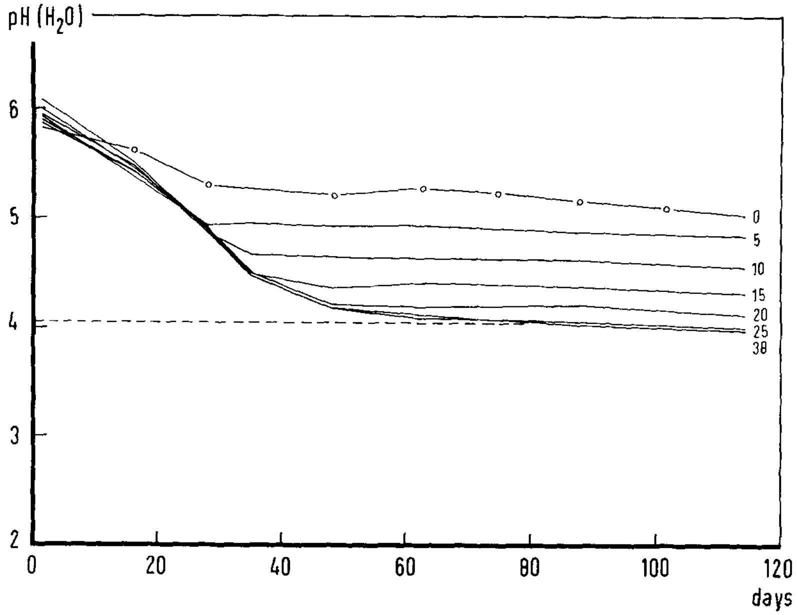

Fig. 2. The change in pH of ----- acidic and —— artificially buffered heathland soil as a function of the amount of ammonium sulphate added (mmol. kg^{-1} dry wt).

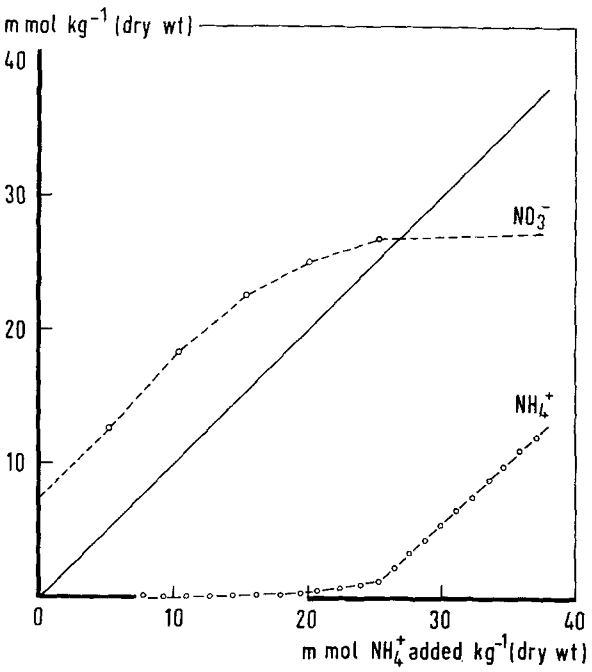

Fig. 3. Ammonium and nitrate concentrations in a moderately buffered heathland soil after incubation for 114 days (20°C) with addition of different amounts of ammonium sulphate.

Table 2. Some nutrient ratios in soil extracts of A: healthy, B: moderately damaged and C: severely damaged *Pinus nigra* var. *maritima* forests. (mol/mol)

		NH_4/K			NH_4/Mg			NH_4/Ca			Al/Ca		
	n	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
A	21	4.7	0.5	14.0	6.4	1.1	24.3	4.0	0.3	9.8	2.0	0.4	5.6
B	17	9.2	0.8	36.8	10.0	1.8	26.3	2.9	0.5	9.2	1.3	0.2	2.8
C	21	11.3	1.9	51.8	22.1	1.6	57.2	10.1	0.7	19.8	5.5	1.7	16.7

Table 3. Potassium, magnesium and nitrogen levels in 1, 2 and 3 years old needles of A: healthy, B: moderately damaged and C: severely damaged *Pinus nigra* var. *maritima* forests (μmol $gr.^{-1}$ dry weight)

Year class	A			B			C		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Potassium									
1	123.8	81.3	150.0	103.2	50.0	128.2	137.0	65.7	325.0
2	112.6	68.8	187.5	43.8	0.0	65.7		Not present	
3	119.4	71.9	215.5		Not present			Not present	
Magnesium									
1	44.1	17.6	80.0	30.1	12.5	51.3	34.1	10.6	55.0
2	33.5	20.0	45.0	22.7	13.1	42.5		Not present	
3	31.8	21.3	56.3		Not present			Not present	
Nitrogen									
1	944	800	1117	1006	844	1478	1269	883	1517
2	878	656	1094	969	544	1561		Not present	
3	828	628	1044		Not present			Not present	

trees (Table 3), but the levels in two year old needles of damaged trees appear to be significantly lower (K: $P = 0.0002$, Mg: $P = 0.036$). The potassium levels and generally the magnesium levels also, are lower than the observed minimum levels in the needles of non-damaged trees. The nitrogen levels in one year old needles of severely damaged trees are significantly higher ($P = 0.0058$).

All trees infected by the fungi *Brunchorstia pinea* (Karst) Höhnel and/or *Diplodia pinea* (Desm.) Kickx had nitrogen levels in the needles exceeding 1150 $\mu mol.g^{-1}$ (dry wt), whereas for all non-infected trees they were below 1150 $\mu mol.g^{-1}$ (dry wt) (Table 4).

Analyses of canopy throughfall and open field precipitation show clear differences in chemical composition (Table 5). In open field precipitation sulphate is fully compensated by ammonium, but only ± 80% in canopy throughfall, the remainder by potassium, magnesium and calcium. Ammonium sulphate levels in canopy throughfall can increase to *ca.* 4000 $\mu mol.l^{-1}$ in areas with high ammonium emission.

The ecophysiological experiments show extensive ammonium uptake by needles and K, Mg and Ca release, when they are incubated

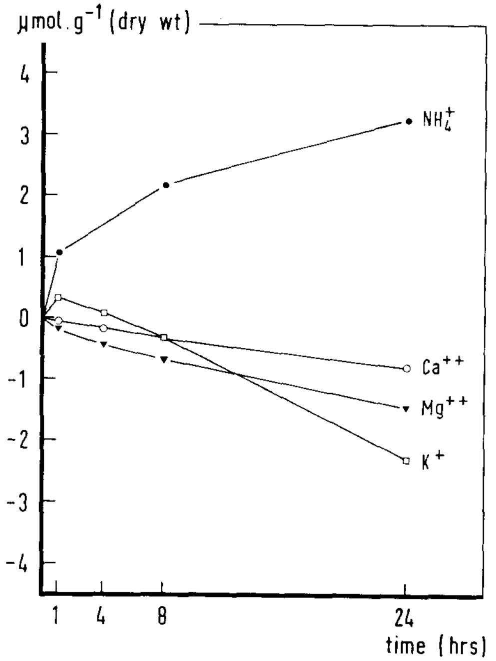

Fig. 4. Ammonium uptake and potassium, magnesium and calcium release from pine needles in a 250 μM ammonium solution.

Table 4. Nitrogen levels in one year old needles of trees infected by *Brunchorstia pinea* (Karst) Höhnel and/or *Diplodia pinea* (Desm.) Kickx and in non-infected trees (μmol gr.$^{-1}$ dry weight)

Infected (n = 11)			Non-infected (n = 17)		
Mean	Min	Max	Mean	Min	Max
1355	1189	1517	956	800	1117

Table 5. The average chemical composition of precipitation in a: open plots (2) and b: throughfall (4) in a *Pinus nigra* forest in an area with intensive stockbreeding (μmol l^{-1})

	NH_4^+	NO_3^-	K^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Na^+	Cl^-	H^+
a	597	71	53	35	71	318	170	197	1.7
b	2283	147	196	94	150	1379	346	462	0.6

in an ammonium containing medium (Fig. 4). This process proceeds continuously. When the needles are added to a medium with a certain salt concentration, rapid excretion of K, Mg and Ca takes place initially, irrespective of whether $(NH_4)_2SO_4$ or Na_2SO_4 has been used. In a medium containing Na_2SO_4 however, the excretion of cations decreases rapidly after a few hours and gradually becomes comparable with that of needles in twice distilled water, whereas in a medium containing $(NH_4)_2SO_4$ the needles continue to excrete relatively

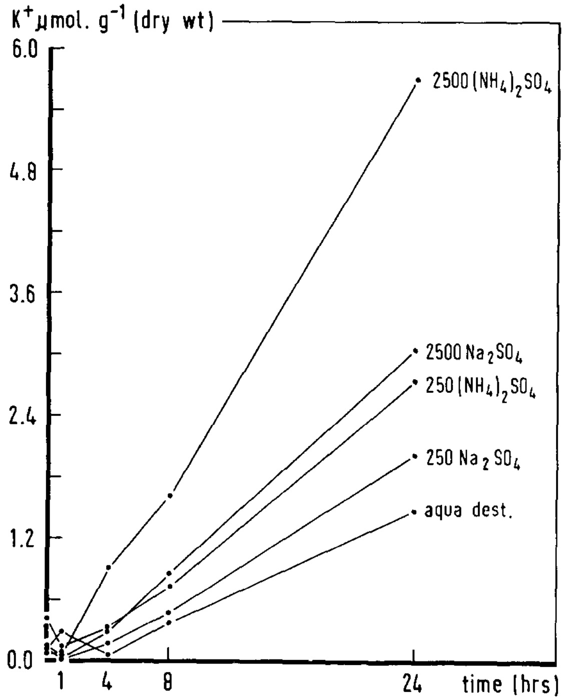

Fig. 5. Potassium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (μM).

large amounts of K, Mg and Ca (Figs. 5, 6, 7). The duplo experiments varied within a few percent. In media with relatively low $(NH_4)_2SO_4$ levels, the excretion of Ca and Mg is more than ten times higher than in distilled water.

Discussion

A clear relation exists between the distance from stock farms and the ammonium sulphate deposition[16]. The deposited ammonium sulphate affects the forest ecosystem. The nutrient release/uptake experiments show that needles of *Pinus nigra* var. *maritima* take up ammonium from $(NH_4)_2SO_4$ solutions and compensate by excreting potassium, magnesium and calcium, which cannot be ascribed exclusively to an osmoregulatory adaptation. This phenomenon has also been described for aquatic macrophytes in acidified $(NH_4)_2SO_4$ containing waters[17]. Forest canopy throughfall analyses suggest a further confirmation of this exchange mechanism. Tissue analyses demonstrate that the observed premature shedding of the needles of damaged trees is related to postassium and/or magnesium deficiencies in older needles. Krause *et al.* also indicate nutrient deficiencies as a result of

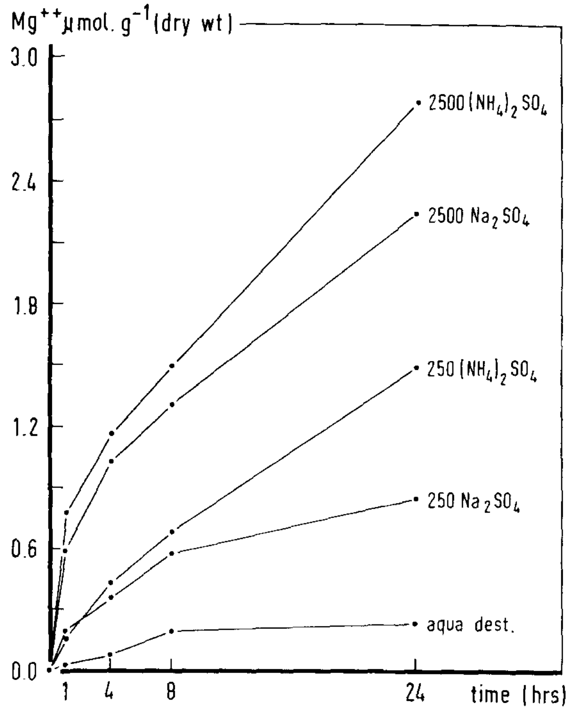

Fig. 6. Magnesium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (*µM*).

cation leaching in German forests, but they ascribe this mainly to needle damage caused by ozone. However, this cation leaching is rather low compared with that caused by ammonium sulphate deposition.

The above mentioned reactions help to explain the high nitrogen levels in pine needles.

All trees investigated and infected by *Brunchorstia pinea* (Karst) Höhnel and *Diplodia pinea* (Desm.) Kickx showed higher total N in the needles. A possible causal relation between high N levels and susceptibility to fungal diseases has to be further investigated.

Several authors[3,4,10,12] mention nitrification of ammonium and the concomitant soil acidification in acidic forest soils. In this study the acidic forest soils investigated seem to show little or no nitrification; the nitrate levels were relatively low and ammonium levels were high. The incubation experiments with artificially buffered soils demonstrated that in these soils nitrification stops or is strongly inhibited at pH (H_2O) < 4.1. Soils of healthy, moderately damaged and severely damaged forests had an average pH (H_2O) of 4.1, indicating that the soil pH probably is determined by the nitrification limit. It should be noted that all the *Pinus nigra* forests investigated had been planted on former sandy acidic heathland soils, so the nitrification results are

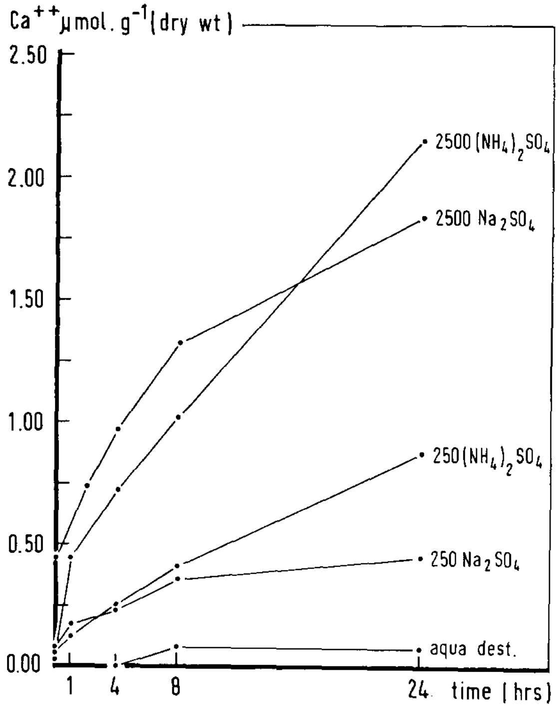

Fig. 7. Calcium release from pine needles in distilled water and in solutions containing ammonium and sodium sulphate (*µM*).

consistent with those of Kriebitzsch[12], who conducted nitrification experiments in many types of acidic forest soil.

The observed ammonium sulphate deposition also results in cation exchange and consequent cation leaching (K, Mg and Ca) from the soil. The NH_4/K, NH_4/Mg and NH_4/Ca ratios are much higher in soils of severely damaged trees. It has already been stated by other authors[7,14] that these increased ratios, especially in soils with a low nutrient level, inhibit nutrient uptake.

The aluminium levels in soils of non-damaged forests are almost equal to those in moderately and severely damaged forests. However, as a result of calcium leaching, the Al/Ca ratio is much higher in soils of severely damaged forests.

Ulrich[20] mentions an Al/Ca ratio > 5 as very critical for root damage. In the soils of severely damaged forests this ratio was indeed above 5, but some of the healthy forests also showed Al/Ca ratios far above this limit. However, according to Ulrich[20] a high Al/Ca ratio does not necessarily result in damage to trees. Organic acids in the soil can detoxify aluminium by forming organic aluminium complexes.

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