Influence of NH_3 and $(NH_4)_2SO_4$ on heathland vegetation

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SUMMARY

Heathland is currently being threatened by nitrogenous atmospheric deposition, mainly NH_y (NH_3 and NH_4^+). *Deschampsia flexuosa* and *Calluna vulgaris* in monocultures were well able to use NH_y for biomass production, although in the case of *C. vulgaris* only shoot growth was stimulated and *C. vulgaris* increased its sensitivity to drought and the heather beetle (*Lochmaea suturalis*). In a mixed culture of *C. vulgaris* and *D. flexuosa*, the latter species was the better competitor for NH_y.

The relation between stimulated biomass production and increased stress sensitivity also applied to several other heathland species.

A general effect threshold for adverse effects of NH_3 on plants has been established with a toxicological model: 270 and 8 $\mu g\ m^{-3}$ for a daily and annual mean, respectively. Heathland species are relatively sensitive to NH_y.

Key-words: ammonia, ammonium, critical level, heathland, stress sensitivity.

INTRODUCTION

The total European emission of NH_3 was $2 \cdot 5\ 10^6$ tons/year in 1870, $3 \cdot 2\ 10^6$ in 1950 and $5 \cdot 2\ 10^6$ in 1980 (Asman *et al.* 1987). Deposition of ammonia and ammonium (together NH_y) is about half of the total nitrogen deposition in many countries (Malanchuk & Nilsson 1989). Background concentrations of gaseous NH_3 and NH_4^+ aerosol are estimated to be less than 1 and 1–3 $\mu g\ m^{-3}$ respectively (Erisman *et al.* 1987), while annual means in regions with intensive livestock farming range from 10 to more than 25 $\mu g\ m^{-3}$. Occasionally and locally, NH_3 concentrations exceed 500 $\mu g\ m^{-3}$. Background concentrations of NH_4^+ in rain water are about 20 $\mu mol\ l^{-1}$, while annual means in regions with intensive livestock farming are about 100 $\mu mol\ l^{-1}$. Concentrations of up to 2000 $\mu mol\ l^{-1}$ in throughfall water under tree crowns in these regions have been measured (Roelofs *et al.* 1985).

N deposition is suspected to be a key factor in the disappearance of plant species (Ellenberg 1985), forest decline (Nihlgard 1985; Encke 1986) and the transition of heathland into grassland (Berendse & Aerts 1984; Berendse *et al.* 1987; Aerts 1989). This paper is restricted to species from heathland, which is a vegetation dominated by evergreen dwarf shrubs, with little or no other shrubs or trees, and with a well developed

moss layer (De Smidt 1982). In the lowlands of Western Europe, naturally occurring heathland is confined to a narrow coastal zone. Inland heath is mostly man-made, although in some localities it may have been in existence for over 500 years.

Syntaxonomically dry heath and transitions between heathland and grassland belong to the class of *Nardo-Callunetea*, characterized by the occurrence of *Calluna vulgaris* (L.) Hull and *Pontentilla erecta* Räusch. (among others). In the transition zones between heathland and grassland, species of the Violion caninae alliance can be found (among others *Antennaria dioica* Gärtn., *Arnica montana* L. and *Viola canina* L.). In the Netherlands and to a lesser extent in surrounding countries, the dominance of dwarf shrubs in the heathland has been changed to one of grasses, strongly dominated by *Deschampsia flexuosa* L. and less often *Molinia caerulea* (L.) Mönch. Species of the Violion caninae alliance become rare. This paper shows direct effects (both primary and secondary) of NH_y on *C. vulgaris*, *D. Flexuosa* and some species of the Violion caninae alliance. An indirect pathway for effects, decomposition of the litter, has been barely considered, although it is very relevant especially for a mature *C. vulgaris* vegetation (Berendse *et al*. 1989; Aerts 1990; Berendse 1990).

In most experiments which aim to simulate the input of atmospheric N deposition in vegetation, N is added directly to the soil, and only once or a very few times per year. In the experiments presented in this paper, nitrogen was supplied through fumigation or artificial rain, allowing both foliar uptake and uptake by the roots. Nitrogen was supplied continuously in the case of NH_3 or every two weeks in the case of $(NH_4)_2SO_4$.

This paper aims to find thresholds for direct, toxic effects of NH_y on heathland species and to evaluate growth responses of *C. vulgaris* and *D. flexuosa* exposed to gaseous NH_3 and artificial rain containing $(NH_4)_2SO_4$. From experiments with nutrient solutions it was concluded that *D. flexuosa* requires relatively low amounts of N for its growth (Hacket 1965; Robinson & Rorison 1983) and can resist high Al concentrations and low pH compared to other grass species (Hacket 1967). This gives it a high competitive capacity on acidic, nutrient poor soils. Competition experiments with *C. vulgaris* and *D. flexuosa* have not been published as yet. In a competition experiment with *C. vulgaris* and *M. caerulea* at N supplies from 0 to 200 kg ha^{-1} year^{-1} over 3 years, Aerts *et al*. (1990) found that even at the highest N supply *C. vulgaris* was not crowded out by *M. caerulea*. They suggested that another, indirect N source is necessary to reach N levels high enough to outcompete *C. vulgaris*: increased biomass production and increased biomass turnover, followed by increased litter production and mineralization (Berendse *et al*. 1989; Berendse 1990). However, the threshold for outcompeting of *C. vulgaris* may be considerably lower in the case of damage to the *C. vulgaris* canopy.

Damage to *C. vulgaris* can be caused, for instance, by an attack of the heather beetle (*Lochmaea suturalis* Thomson), drought or frost. Regenerative capacity following damage can be inhibited at high N supplies (Berdowski 1987; Van de Eerden *et al*. 1990). Heather beetle outbreaks occur occasionally in Dutch heathlands (Berdowski & Zeilinga 1987). *L. suturalis* prefers leaves with a high nitrogen concentration (Heil 1985; Berdowski & Zeilinga 1987). Therefore, a positive correlation between exposure of *C. vulgaris* to NH_3 and the growth of larvae was hypothesized in our experiment. In this paper the influence of NH_3 is evaluated on a relatively short period of the entire life span of *L. suturalis*. The impact of NH_3 during other periods (e.g. survival during winter) may be relevant as well.

Influences of NH_3 on frost and drought tolerance (on species other than *C. vulgaris*) are reported by Dueck *et al*. (1991).

If stress sensitivity of *C. vulgaris* increases with high N deposition, this sensitivity is a relevant parameter for defining effect thresholds.

Establishing effect thresholds is necessary in order to provide standards for environmental policy. To express thresholds in deposition terms such as kg N ha^{-1} year^{-1} is most common. But from the phytotoxicological point of view discrimination between N species (NO, NO_2, NO_3^-, NH_3, NH_4^+) is useful (e.g. Raven 1988). Expressing thresholds for atmospheric N pollution not only in deposition terms (critical loads), but also in terms of atmospheric concentrations (critical levels) is relevant if direct and short-term effects are to be expected and foliar uptake is not neglectable. This may be the case with NH_3. Two methods to estimate a general effect threshold for the impact of gaseous NH_3 on plants are discussed.

MATERIAL AND METHODS

Experimental conditions

The soil in which the plants were grown was sandy, nutrient-deficient (total-N content: 0·85 mg g^{-1}) and acidic (pH($CaCl_2$): 3·7). Plants were collected from the field except for species of the Violion caninae alliance which were grown from seeds.

Atmospheric NH_3 concentrations were measured with a chemiluminescent NO_x monitor (Monitor Labs, 8840), preceded by a thermoconvertor (Monitor Labs, 8750). SO_2 was measured with a fluorescent SO_2 analyser (TECO, 43). Gases were bled into the ventilation air of the fumigation chambers, using thermal mass flow controllers (Brooks).

Experiments were carried out in four different facilities.

1. Experiment A was carried out in stainless steel fumigation chambers with a ground area of 1·65 m^2 in which temperature, relative humidity and light intensity were computer controlled. Air velocity was 0·85 m sec^{-1}. Inlet air was filtered by activated charcoal (contact time 1·5 sec). During the day (8–19 h) temperature, relative humidity and light intensity were 17°C, 75% and 70 W m^{-2}, respectively. The light was supplied by HPL-N (mercury) and SON-T (sodium) lamps. At night conditions were 11°C, 90% and 0 W m^{-2}.

Hypnum jutlandicum Holmen & Warneke, *Racomitrium lanuginosum 1 (Hedw.) Brid.*, *Pleurozium schreberi* (Brid.) Mitt., *Campylopus flexuosus* (Hedw.) Brid., *C. vulgaris* (L.) Hull, *Agrostis capillaris* L., *Antennaria dioica* (L.) Gaertn., *Potentilla erecta* (L.) Räusch. and *Viola canina* (L.) were fumigated for 90 days with NH_3 concentrations ranging from 0 to 240 μg m^{-3}. All species were judged on visual injury. From all species except bryophytes, shoot and root dry weight was measured after drying for 48 h at 75°C. To indicate drought stress the water potential of *C. vulgaris* shoots was measured with the pressure bomb technique (Scholander *et al.* 1965) at the end of the fumigation, after a dry period of 6 to 10 days. Water potential of the drought treated plants was compared with plants which were supplied with sufficient water (six replicates per treatment).

2. Experiment B was executed in fumigation greenhouses with a ground area of 12 m^2 in which temperature was controlled. Light was partly from outside and partly artificial (multivapour lamps; 15 W m^{-2} at plant height). Air velocity was 0·15 m s^{-1}. Inlet air was filtered by activated charcoal (contact time 0·1 sec). During the day (8–19 h) temperature and relative humidity were 20°C and 44%, respectively. At night they were 15°C and 56%. Light intensity was partly determined by ambient light, causing a seasonal variation: around 50 W m^{-2} in the first and last 10 weeks of the exposure period and around 25 W m^{-2} in the middle 18 weeks, which was winter time. *C. vulgaris* and *D. flexuosa* were treated with air containing 100 or less than 10 μg m^{-3} NH_3. Every month five plants were

harvested and biomass and N content was measured after 48 h drying at 75°C. N content was measured by column chromatography after combustion in O_2 and He at 1000°C and reduction over Cu (Carlo Erba Elemental Analyser).

3. Experiment C took place in open-top chambers with a ground area of 9·6 m². The chambers were ventilated with ambient air without filtration or climate conditioning. Air velocity was 1·5 m sec⁻¹. On average, the temperature was less than 1°C higher than ambient temperature. Due to the constant air flow (85 m³ min⁻¹) dew rarely developed and water use of the plants was greater compared with conditions in the field. In a period of 18 months, experiments with different exposure lengths were performed. The winter in the middle of this period was mild, with only very few days of frost and the subsequent spring was extraordinarily dry (precipitation of 90 instead of 180 l m⁻² in 3 months). The plant species in this experiment were *C. vulgaris*, *D. flexuosa*, *A. dioica*, *A. montana* and *V. canina*. They were fumigated with NH_3 with concentrations ranging from 3 to 107 μg m⁻³. In one NH_3 treatment 90 μg m⁻³ SO_2 was added.

The influence of NH_3 on the growth of *L. suturalis* was studied following a 12 month fumigation of *C. vulgaris* at four NH_3 concentration levels. Larvae were collected from the field. On each of five plants per treatment 15 larvae (five per instar) were fed for 7 days with shoots from fumigated plants. Growth was indicated by the ratio of number of larvae in the third instar and the total number of larvae.

4. Experiment D was artificial rain experiment and was performed under a plastic roof measuring 54 m². Light transmission was reduced by the roof by 25%. Dry deposition of gaseous air pollutants was not influenced by this roof (Bobbink *et al.* 1990). Once every two weeks, artificial rain (30 l m⁻²; 1 l min⁻¹ m⁻²) was sprayed with 10 nozzles per m² over a mixed vegetation of *D. flexuosa* and *C. vulgaris* in containers (50 l each), filled with soil. The artificial rain water consisted of demineralized water to which sea salt and HCl was added up to a Cl concentration of 80 μmol l⁻¹ and a pH of 5.6. The $(NH_4)_2SO_4$ concentrations were 20, 100, 200 and 400 μmol l⁻¹.

The vegetation had an adaptation period of 2 months before the treatment started. Biomass of each species was about 30 g at the start of the experiment and each species covered approximately one-third of the soil surface in the container. The experiment started in early summer and lasted 13 months. At final harvest, shoot and root biomass of both species were measured. To have an indication for light interception of *D. flexuosa* above *C. vulgaris* the shoot weight of *D. flexuosa* was divided in portions above and below 25 cm.

Estimation of effect thresholds

An effect threshold for gaseous NH_3 was estimated with two different methods. The first is discussed by Posthumus *et al.* (1989): in a concentration/time graph a curve is fitted directly below the lowest effective exposure levels. The second is a toxicological model, described for water pollution by Kooijman (1987) and for soil pollution by Van Straalen & Denneman (1989). This model calculates a safety factor based on deviation in sensitivity between species and the number of evaluated species. Input data are 'No Observable Effect Concentrations' (NOECs). The NOECs of different species within a population are assumed to be ln-normally distributed. The mean NOEC is calculated as the mean of the ln-transformed NOECs of the tested species. The standard deviation of this mean is used to arrive at a safety factor(T). Dividing the geometrical mean of the NOECs by T yields a concentration at which a certain fraction (1-*d*) of the species in a community is protected

against 'observable' adverse effects. For example: $d = 0.05$, implies that T is calculated to aim a protection of 95% of the species.

T can be calculated as:

$$T = \exp\left[(3s_m D_m/\pi^2) * \ln(1-d)/d\right]$$

with:

s_m standard deviation belonging to the ln NOEC
D_m factor dependent on number of tested species, calculated according to Kooijman (1987) (e.g. 3.06, 2.59 and 2.25 for 5, 10 and 50 species, respectively if $d = 0.05$)
d fraction of species that is not protected by the calculated safety factor

Data presented in this paper, combined with literature data on NH_y effects (Van der Eerden 1982; Van der Eerden et al. 1990) were evaluated with both the method of Posthumus et al. (1989) and that of Kooijman (1987). An effect threshold specifically for heathland vegetation was not estimated, because the amount of available relevant data was too small. To establish effect thresholds in a limited number of exposure periods, the data were interpolated to 1 h (for the interval of < 10 h), 1 day (for 10–99 h), 1 month (100 – 999 h) and 1 year (> 1000 h), using the formula:

$$(1 + \log t)(Q + \log C) = 4.0089$$

where t is in hours, C in $\mu g \, m^{-3}$ and Q is a species dependent value (Van der Eerden 1982).

Experimental data on the relation between NH_3 (concentration and exposure time) and growth response of C. vulgaris, D. flexuosa and L. suturalis, and of the drought stress of C. vulgaris after fumigation with NH_3 were tested using regression analysis after ln-transformation where relevant (Lindgren & McElrath 1969). Differences in response per treatment level of NH_3 or $(NH_4)_2SO_4$ were compared by analysis of variance after testing for homogeneity of variances and followed by Students t-test where necessary.

RESULTS

Experiment A

Table 1. Lowest NH_3 concentrations ($\mu g \, m^{-3}$) at which bryophytes first showed leaf tip chlorosis 11, 14, 17 and 23 days after exposure to NH_3. The highest concentration was 240 $\mu g \, m^{-3}$; > 240 indicates that no injury was observed

Exposure time:	11	14	17	23 days
Species:				
Pleurozium schreberi	> 240	> 240	> 240	> 240
Campylopus flexuosus	> 240	120	120	120
Hypnum jutlandicum	120	120	120	120
Racomitrium lanuginosum	> 240	120	60	30

The first visible effect in this NH_3 fumigation was on bryophytes: leaf-tip chlorosis, followed after a few days by necrosis. The response was strongly species dependent (Table 1). The higher plant species showed growth stimulation, mostly more shoot than root

Table 2. Linear regression of shoot and root biomass (g dwt) related to ln-transformed NH_3 concentrations after a 90 days exposure. Significance of the slope is indicated with P-levels. Estimated NH_3 concentrations ($\mu g\ m^{-3}$) for 50% stimulation of shoot growth is indicated

	Intercept	Slope (sign)	Percentage variance accounted for NH_3	NH_3 concentration for 50% growth stimulation
Species				
Calluna vulgaris				
shoot	1·85	0·195 ($P<0·001$)	67	41
root	3·11	−0·074 (NS)	3	
Potentilla erecta				
shoot	−0·123	0·204 ($P<0·001$)	49	145
root	−1·02	−0·009 (NS)	0	
Viola canina				
shoot	−2·22	0·286 ($P<0·001$)	66	21
root	−0·480	0·113 ($P<0·01$)	16	
Agrostis capillaris				
shoot	−0·606	0·255 ($P<0·001$)	54	13
root	−0·480	0·164 ($P<0·01$)	18	
Antennaria dioica				
shoot	0·881	0·179 ($P<0·001$)	59	49
root	1·30	0·176 ($P<0·001$)	40	

growth. In the case of *C. vulgaris* and *P. erecta* roots were not significantly influenced, while shoot growth of *V. canina* and *A. capillaris* was stimulated about double as much as root growth. Only *A. dioica* had equal stimulation of shoot and root (Table 2).

Drought tolerance of *C. vulgaris*, indicated by the water potential of its shoot, was tested following the fumigation. Figure 1 shows a more negative water potential with increasing NH_3 concentration and increasing length of dry periods. The water potential values declined only after several days of drought and more severely as the NH_3 concentration increased. Following most of the drought treatments the plants recovered, except at the highest NH_3 concentrations combined with 10 days of drought. At that combination treatment, most of the plants showed severe drought injury, which obviously is the cause of some of the extremely low water potential levels.

Experiment B

The NH_3 fumigation in experiment A aimed to study the effect of NH_3 over a range of concentrations for a fixed exposure period. Experiment B was performed to study the effect of a fixed concentration level (100 $\mu g\ m^{-3}$), for various exposure lengths.

The positive effect of NH_3 on total dry weight, leaf dry weight and total amount of N (N concentration multiplied with plant dry weight) in *C. vulgaris* and *D. flexuosa* is shown in Figure 2. Towards the end of the fumigation period the total amount of N did not increase anymore. At that moment the gain in N content due to NH_3, relative to the content at the start of the fumigation, was significantly ($P<0·01$) higher in *C. vulgaris* than in *D. flexuosa*: 403 and 283%, respectively. Biomass of the leaves and the total plant increased, although an exponential phase of growth stimulation may have lasted for only
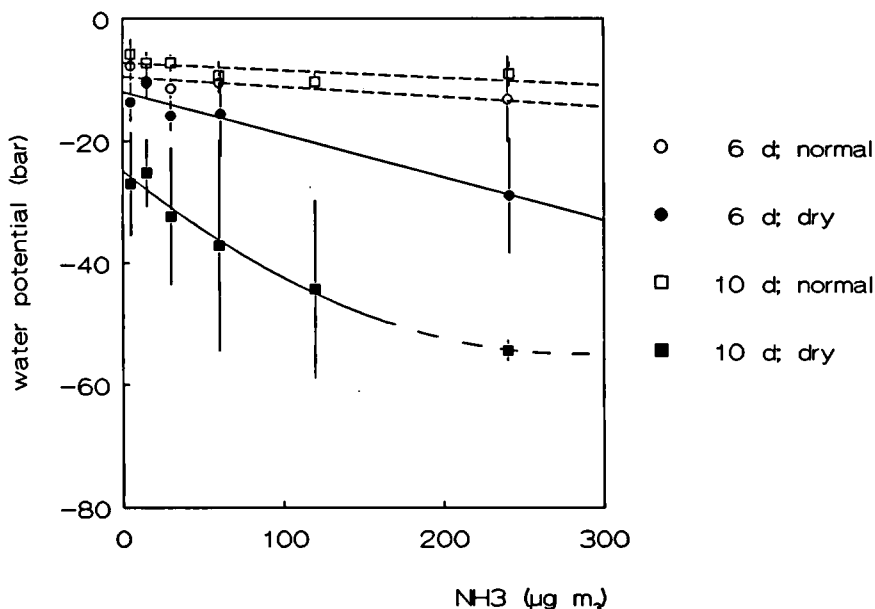
Fig. 1. Influence of a 60-day exposure to a range of NH_3 concentrations, followed by either dry periods of 6 and 10 days (solid lines) or sufficient water supply during the same periods (broken lines), on the water potential of the shoot of *Calluna vulgaris*. Bars indicate standard deviations; $n = 5$.

about 2/3 of the exposure period. Expressed in percentage of the level at the start of the fumigation and using a fit with an exponential curve, the species differences in response to NH_3 were relatively small. After 25 weeks for instance, the increase in total biomass due to NH_3 was 91% and 131% for *C. vulgaris* and *D. flexuosa*, respectively (not significant). At that moment the difference in stimulation of leaf biomass was larger: 140% and 291% for *C. vulgaris* and *D. flexuosa*, respectively (significant at $P < 0.01$).

Figure 3 shows the effect of NH_3 on the root/shoot ratio for both species. In the case of *C. vulgaris* the effect of NH_3 was mainly stimulation of the shoot (significant at $P < 0.05$ for exposure periods of 9 weeks and longer). This was also true for *D. flexuosa*, but only 3 and 13 weeks of exposure: after this period the differences in root/shoot ratio of fumigated and non-fumigated plants were insignificant.

Experiment C

The development of *L. suturalis* larvae fed on NH_3 fumigated *C. vulgaris* was tested in this experiment. On average, only 74% of the larvae survived the test. This percentage was not related to the NH_3 concentration. Although more larvae of the third instar were found on *C. vulgaris*, exposed to higher NH_3 concentrations $P < 0.01$; (Fig. 4), the percentage of variance accounted for this NH_3 treatment was only 26%.

Besides *C. vulgaris*, four other heathland-species were fumigated. In the charcoal filtered and unfiltered air treatments (3 and 6 µg m^{-3} NH_3, respectively) most of the plants survived the entire experiment, including the winter and the following, extraordinarily dry spring. Some mortality however, did occur in the higher NH_3 concentrations. The group that survived continued growing and started flowering. For *C. vulgaris*, *A. montana* and *A. dioica*, (in contrast to *D. flexuosa* and *M. caerulea*) the
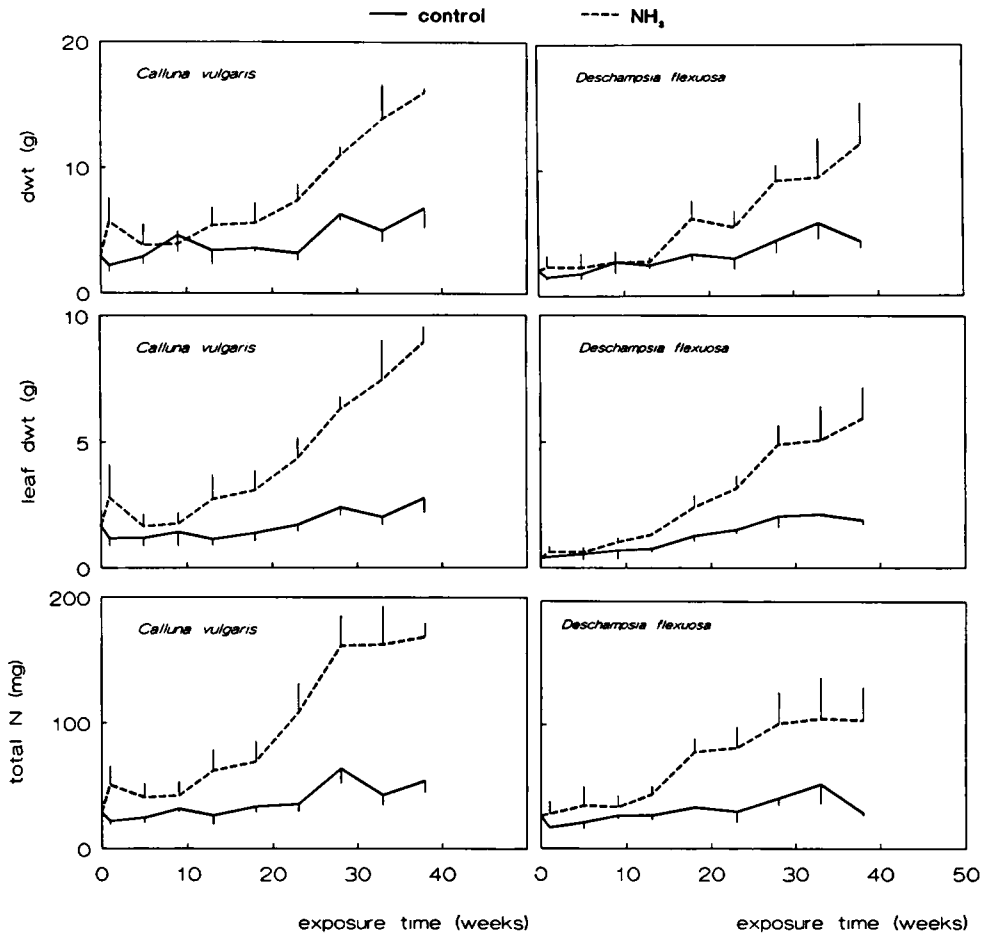
Fig. 2. Total biomass (upper two figures), leaf biomass (middle two figures) and N content (lower two figures) of *Calluna vulgaris* (left set of three figures) and *Deschampsia flexuosa* (right set of three figures) during a 38 week fumigation with 100 μg m⁻³ NH₃. Bars indicate standard deviations; $n = 5$.

percentage of survival depended on the NH_3 concentration (Table 3). The combination of stimulated vegetative growth and decreased flowering and survival of *A. montana* is shown in Figure 5.

Experiment D

A mixed vegetation of *C. vulgaris* and *D. flexuosa* was treated with artificial rain, containing ammonium sulphate in four concentrations. Biomass per container increased considerably during that period from 30 g for both species at the start of the experiment to about 1·5 kg for *C. vulgaris* and, depending on the treatment, two to three kg for *D. flexuosa*. The vegetation completely covered the soil in all containers at harvest. In the case of *D. flexuosa* a positive correlation was found between the biomass production and the ammonium sulphate concentration in artificial rain. (Fig. 6). The $(NH_4)_2SO_4$ treatment accounted for 47% of the variance for both shoot and root growth of *D. flexuosa*. No clear treatment effect was found for *C. vulgaris*, although the results show
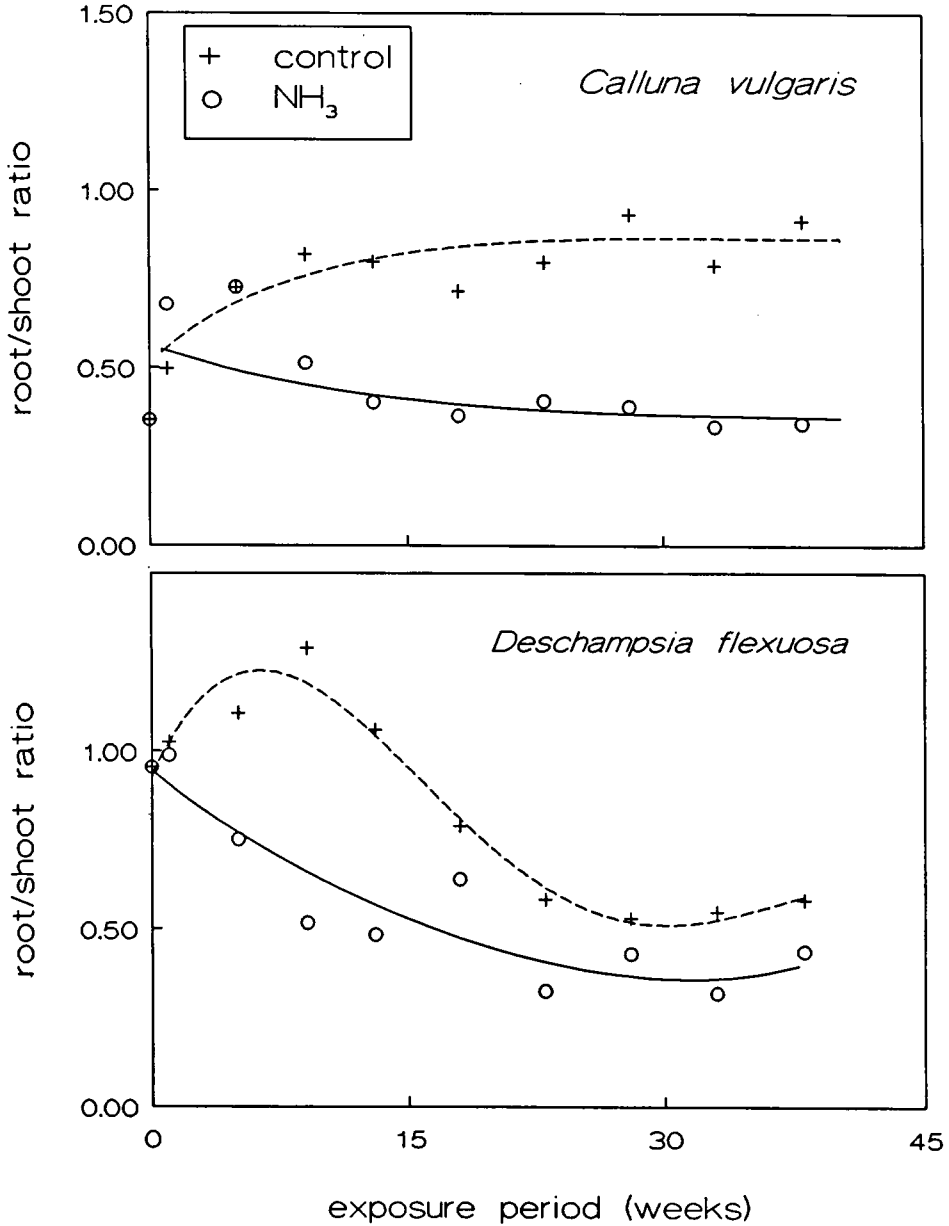
Fig. 3. Root/shoot ratio of *Calluna vulgaris* and *Deschampsia flexuosa* following a 38 week fumigation with $100 \, \mu g \, m^{-3} \, NH_3$; $n = 5$.

some tendency of depressed root growth. Early in the second growing season, *D. flexuosa* produced long panicles, which intercepted some of the light above *C. vulgaris* (Fig. 7).

DISCUSSION

Although the primary effect of NH_3 in concentrations lower than $100 \, \mu g \, m^{-3}$ is growth stimulation, rather than toxic effects (Van Hove *et al.* 1989; Van der Eerden *et al.* 1990;
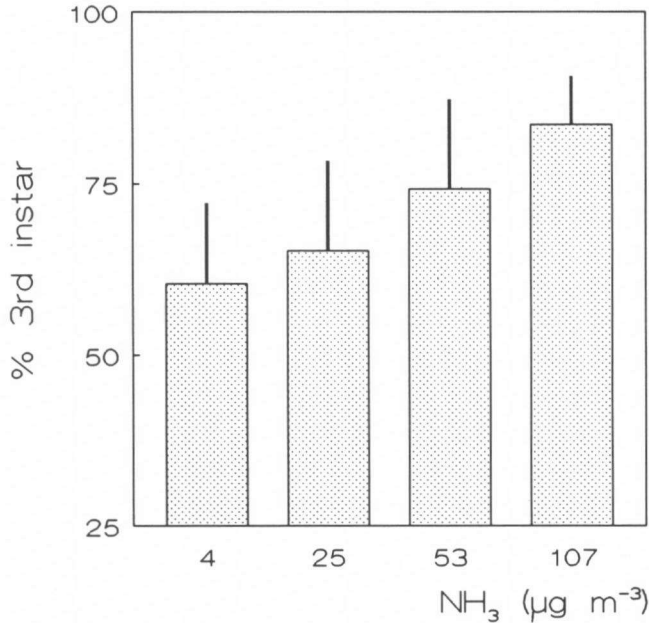
Fig. 4. Growth of *Lochmaea suturalis* larvae, feeding on shoots of *Calluna vulgaris* that were exposed to NH_3 for 12 months. Growth was determined after 7 days and is indicated by the ratio of the number of larvae in the third instar and the total number of larvae. The lines above each bar indicate standard deviations ($n = 5$).

Table 3. Survival (%) of several species from a heathland vegetation following a fumigation with NH_3 for 16 months. The indicated NH_3 concentrations are in unfiltered air, except for $3\,\mu g\,m^{-3}$, which was in air filtered with activated charcoal

NH_3 ($\mu g\,m^{-3}$)	3	6	35	53	105
Antennaria dioica	70	100	60	30	30
Arnica montana	100	70	60	50	50
Calluna vulgaris	73	93	73	67	50
Deschampsia flexuosa	100	100	100	100	100
Molinia cearulea	100	100	100	100	100

Table 2) some bryophyte species may be an exception. One species out of four showed leaf injury at $30\,\mu g\,m^{-3}$ after 23 days (Table 1). The relatively high sensitivity of bryophytes may be due to the high permeability of their cuticula. Other tested species showed growth stimulation when no other stress factors were involved. Monocultures of both *C. vulgaris* and *D. flexuosa* were able to use NH_3 for biomass production up to exposure levels that considerably exceed ambient concentrations in polluted regions. The hypothesis that *D. flexuosa* responds much faster to NH_3 than *C. vulgaris* and that *D. flexuosa* is the better N accumulator of the two, could not be confirmed on the basis of experiment C ($100\,\mu g\,m^{-3}$ NH_3 for 38 weeks). With prolonged exposure, a level may have been reached where N cannot be used anymore for biomass production and further N uptake is either luxury consumption or toxic. Other nutrients can become growth limiting. But despite of the high
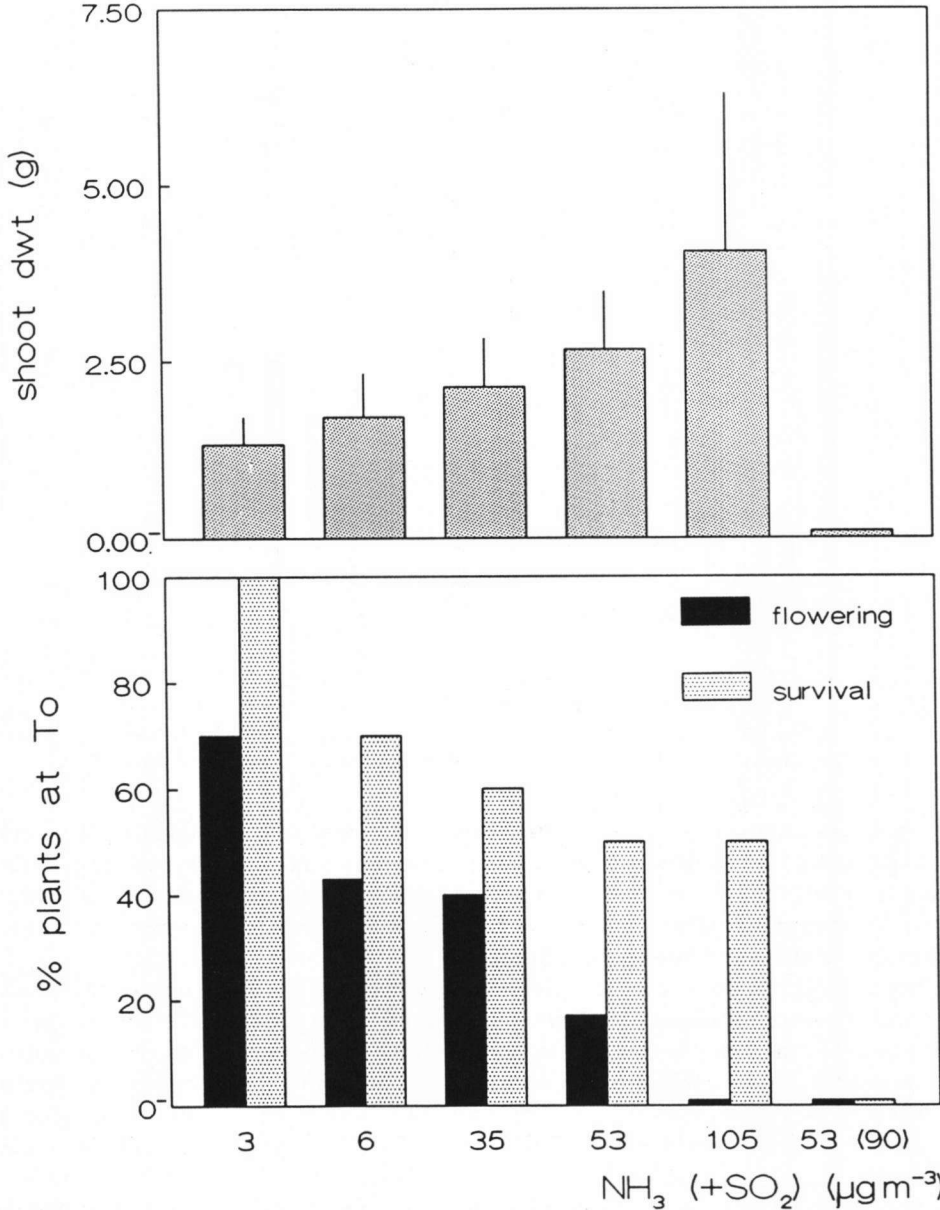
Fig. 5. Influence of a 16 month exposure to NH_3 on shoot growth (upper figure) and flowering and survival (lower figure) of *Arnica montana*. The indicated NH_3 concentrations are in unfiltered air, except for 3 μg m^{-3}, which was in air filtered with activated charcoal. The lines above each bar in the upper figure indicate standard deviations ($n = 10$).

exposure level, this threshold was not reached apparently in this fumigation. This is in agreement with the results of Hacket (1965), who found for *D. flexuosa* a low N requirement for biomass production (but no toxic level for NH_4) and no growth response over wide range of Ca, Fe, Mn and K supply.

A relationship was found between increased biomass production during the growing season and decreased survival after a winter and a (relatively dry) spring. This applied to
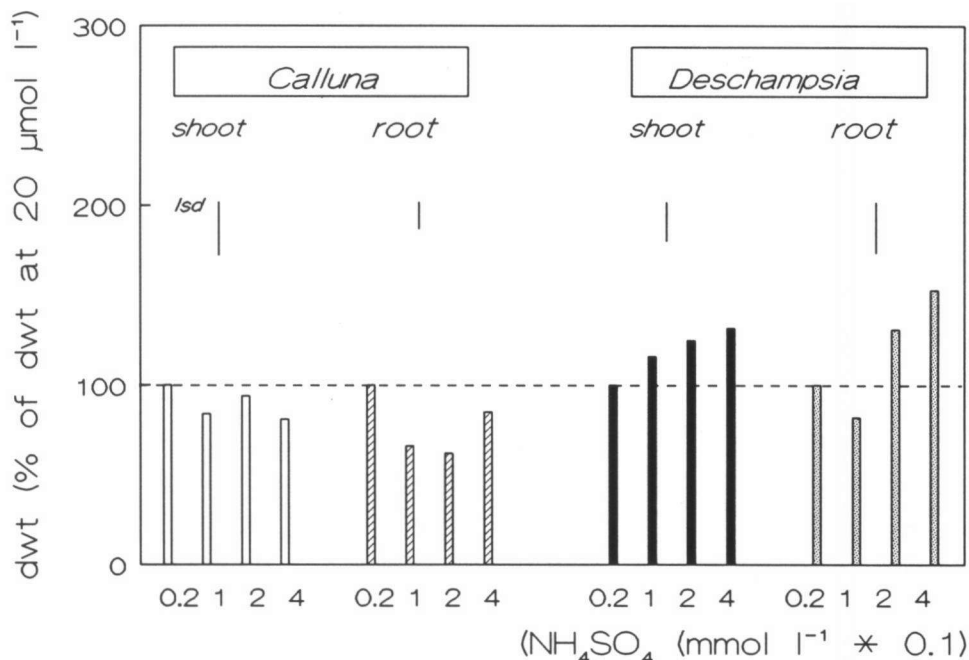
Fig. 6. Influence of artificial rain, supplemented with 20, 100, 200 and 400 μmol l^{-1} (NH$_4$)$_2$SO$_4$ on the biomass production of *Calluna vulgaris* and *Deschampsia flexuosa*, expressed as percentage of the biomass at 20 μmol l^{-1}, $n=4$. Vertical lines above each set of four bars indicate the least significant difference at $P=0.05$.

C. vulgaris and even more to species of the *Violion caninae* alliance (Table 3). In relation to this experiment, the following should be noted: the effects of NH$_3$ on survival are probably stronger in this fumigation, compared with the field situation, because the plants were grown for 16 months in plastic pots in open-top chambers where desiccation occurs much more rapidly compared with field conditions.

That growth responses can be different in the case of competition compared with monoculture is well known, and was also found in experiment D. *C. vulgaris* in monoculture was well able to use NH$_u$ for its biomass production, but not in a mixed culture with *D. flexuosa* (Fig. 6). One reason for this phenomenon might be the difference in timing of the growth response. In experiment D *D. flexuosa* started growing earlier in the growing season and intercepted light above the *C. vulgaris* vegetation. In mixed cultures of *C. vulgaris* or *Eric tetralix* with *M. caerulea*, Aerts *et al.* (1990) found light interception to be the dominant factor influencing competition. This may be true for the combination of *C. vulgaris* and *D. flexuosa* as well. In our experiment, light interception appeared to be greater at higher (NH$_4$)$_2$SO$_4$ concentrations. A threshold for this effect may exist between 100 and 200 μmol l^{-1}, rather than a linear relation between treatment and response (Fig. 7).

Although NH$_y$ certainly influences the competitive ability of *C. vulgaris*, this is probably not the only reason for the transition of heathland into grassland. Competition for space, nutrients and light takes place only at the edge of gaps with *D. flexuosa*, in a closed *C. vulgaris* vegetation. The expansion of these gaps continues until *C. vulgaris* is crowded out and takes some decades if this is not stimulated by other factors as well. In the Dutch field situation transition of heathland into grassland often occurs within one or two
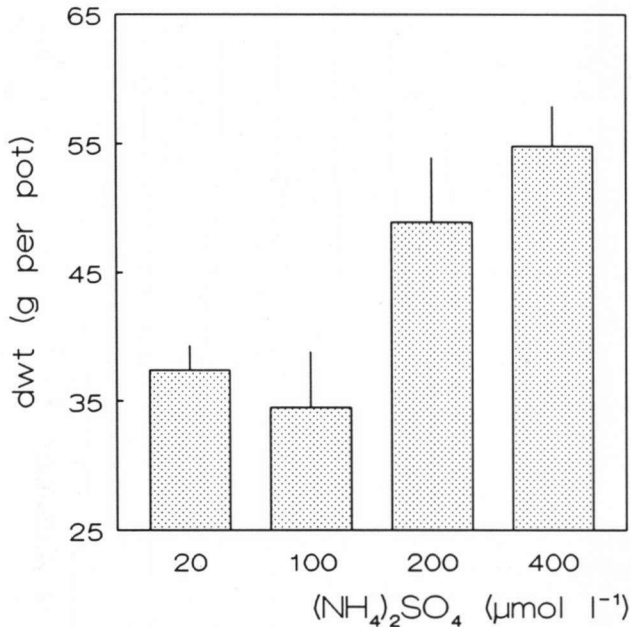
Fig. 7. Influence of artificial rain, supplemented with 20, 100, 200 and 400 μmol l^{-1} (NH$_4$)$_2$SO$_4$ on the biomass production of *Deschampsia flexuosa* (g dwt per container) growing above the *Calluna vulgaris* canopy. Vertical lines above each bar indicate standard deviations; $n = 4$.

years. Therefore, other factors inhibiting the *C. vulgaris* vegetation are necessary to explain this process. Two factors, drought and a plague of *L. suturalis*, were evaluated in this paper. To some extent, these stress factors are a constant threat to the *C. vulgaris* vegetation, but our results suggest that the sensitivity of *C. vulgaris* slightly increases due to exposure to NH$_y$.

A decreased root/shoot ratio (Table 2 and Fig. 3) is probably one of the reasons for increased drought sensitivity of *C. vulgaris* after exposure to high NH$_3$ concentrations (Fig. 1). An additional reason for the increased drought sensitivity may be that NH$_y$ stimulates photosynthesis and transpiration (Van der Eerden *et al.* 1990). After some months of exposure to NH$_3$ *D. flexuosa* appeared to bring its root/shoot ratio close to that of the plants in the control treatment. This indicates a high degree of plasticity of *D. flexuosa* in relation to nitrogen supply, which is in agreement with the results of Robinson & Rorison 1983, 1988).

Some indication of an increased rate of development of the larvae of *L. suturalis* larvae was found after consuming *C. vulgaris* shoots fumigated with NH$_3$. Significantly more larvae of the third instar were found in the higher treatment levels. The larvae reach their maximum weight and are most harmful in the third instar. However, the variation is large (the variance accounted for the NH$_3$ treatment was 26%) and the slope in the relation between larval development and NH$_3$ concentration was very slight:

$$\%3\text{rd} = 54 \cdot 1 + 0 \cdot 0306 \, \text{NH}_3$$

where '%3rd' is the proportion of third instar larvae and NH$_3$ is in μg m^{-3}.

In most of the experiments described here, a more or less linear relationship between NH$_y$ and biomass production was found. This suggests that in polluted areas in the
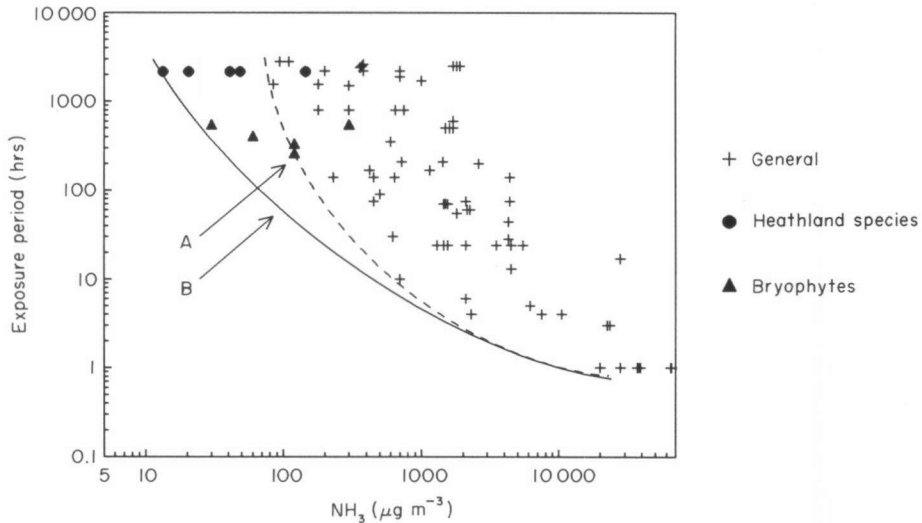
Fig. 8. Summary of adverse effects of NH_3 on several plant species at several combinations of time and concentration. Triangles mark bryophyte species from a heathland vegetation and dots mark other heathland species. The solid curve (B) is drawn below all effective exposure levels, while the broken curve (A) neglects the heathland species.

Netherlands (annual means of 10–20 µg m^{-3} NH_3 and 100 µmol l^{-1} $(NH_4)_2SO_4$ in rain water) an increase of 10–25% in biomass per growing season can be expected in most heathland species, compared with unpolluted regions. Direct injury of some bryophytes can occur at ambient concentrations. Although sensitivity of *C. vulgaris* to drought and *L. suturalis* increased due to NH_3, the effective exposure levels were relatively high and the slope of these relations is very low, indicating a minor effect close to ambient NH_3 concentrations.

Effect thresholds

An attempt was made to determine effect thresholds for NH_y. Not enough data are available to define an effect threshold for NH_4^+. In the case of NH_3 much more data are available, but here too some simplification was needed. Interactions with other pollutants and delayed exposure due to temporary immobilization in the litter layer are neglected.

The effects mentioned in this paper have been used to arrive at a threshold for adverse effects. Adverse effects in this case are tissue injury for bryophytes (Table 1) and growth stimulation of 50% or more for the other species (Table 2). These data complemented literature data on adverse effects (mainly leaf injury) to other plant species (Van der Eerden 1982) and are summarized in Figure 8. Some of these effects must be considered as indicators for an adverse impact of the pollutant, rather than a quantitative estimation of the response on ecosystem level. The use of this type of indications is common in setting standards for air quality in respect with the effects of air pollution on vegetation (Posthumus *et al.* 1989).

This data set can be used to arrive at effect thresholds in two ways. One is the 'graphical' approach (Posthumus *et al.* 1989). Figure 8 shows that the effects of NH_3 presented in this paper cause a decrease of the no-adverse-effect level. A general effect threshold derived from the left curve in Figure 8 is in the order of magnitude of 10 and 250 µg m^{-3} for

Table 4. Critical levels for adverse effects of
NH_3 on plants, based on data summarized in
Figure 8 and model calculations. At these
levels 95% of the species are protected

1 hour	3300 μg m^{-3}
1 day	270
1 month	23
1 year	8

exposure periods of 1 year and 1 day, respectively. Figure 8 shows that for exposure periods of more than 10 days, heathland species (including bryophytes) determine the general effect threshold. However, quantitative interpretation of this curve is dubious, due to the very limited amount of effective exposure levels which are relevant for drawing the curve.

A second approach to arrive at effect thresholds is to use the model of Kooijman (1987) and Van Straalen & Denneman (1989). Under certain conditions the entire data set, shown in Figure 8, can be used. One condition is that the distribution of the input data approaches log-normality and this holds for the data of Figure 8. Another condition is that the data are representative for the entire population, which is the entire plant community in the case of a general effect threshold. This may not be entirely true for the data set of Figure 8, but other data are currently not available.

A general effect threshold, calculated with this model is given in Table 4. Although the average sensitivity of heathland species is relatively high, evaluation of only heathland vegetation results in a much lower standard deviation. (s_m in the model) which strongly compensates for the lower average NOEC. A specific effect threshold for heathland vegetation may not be very different from a general effect threshold. But this suggestion can only be tested on the basis of more data.

The ambient concentrations in several regions in Western Europe are well above the effect thresholds, mentioned in Table 4. The relevance of this excess is difficult to interpret in all details as yet. But indications of a serious impact of NH_y on vegetations are obvious.

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