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Critical Loads of N & S Deposition to Semi-Natural Vegetation

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1 EXECUTIVE SUMMARY

This report describes part of an integrated research programme with the Universities of Manchester and Newcastle-upon-Tyne to determine critical loads and levels of deposited sulphur and nitrogen to semi-natural ecosystems in the UK. The role of ITE Bangor has been to determine the effects of gaseous and wet forms of combined sulphur and nitrogen on the growth and physiological responses of plants under controlled conditions of exposure, during a three year programme.

The first phase of the research aimed to determine whether critical levels and loads of individual pollutants for native plant species are altered by the presence of other pollutants. A series of studies assessed a) the separate and interactive effects of ozone and acidic gases (SO_2 + NO_2) b) the effects of acid mists at a range of pHs and c) the interactive effects of gaseous pollutants and acid mists on the growth responses of a range of native plant species.

The data clearly demonstrated that species differed substantially in their sensitivities to the various pollution treatments. While some species, notably the two leguminous species studied, showed decreased dry matter production in highly acidic mist treatments compared to control plants (Ph 5.6 mist) others showed increased growth. Similarly, while some of the species studied were unaffected by exposure to the pollutant gases, others suffered substantial reductions in biomass in all air pollution treatments compared to plants grown in charcoal-filtered air. There was some evidence that the presence of ozone could affect critical levels/loads of SO_2 + NO_2 for some species. Studies of combined mist x gas exposures revealed that critical loads/levels of S + N are substantially different for gaseous as opposed to wet inputs to vegetation. While high inputs (60 – 70 $kg.ha^{-1}$ S + N) as wet deposition increased biomass in some species, similar levels and much lower inputs caused substantial reductions in biomass in the presence of SO_2 + NO_2 gases.

Phase two of the programme aimed to establish dose–response relationships for species to SO_2 + NO_2 exposures. Six of the ten species studied showed no effects on growth of concentrations up to 40 ppb SO_2 + 40 ppb NO_2. Three species showed a growth stimulation at 10 ppb SO_2 + 10 ppb NO_2 and 20 ppb SO_2 + 20 ppb NO_2 in comparison to plants grown in charcoal filtered air and ones exposed to 40 ppb SO_2 + 40 ppb NO_2. The tenth species showed a growth stimulation at 20 ppb SO_2 + 20 ppb NO_2 compared to all other treatments. These results indicate a potential fertilizer effect of the pollutant combination at low (ambient) levels. However, once again effects were not related to length of exposure or 'Ellenberg'nitrogen values and there was no consistent difference in response of calcareous v acidic grassland species.

The final phase of the research programme aimed to determine dose–response relationships to inputs of SO_2 + NO_2 for a typical calcareous grassland species (*Briza media*) and an acidic grassland species (*Agrostis capillaris*) over a range of additions of wet combined nitrogen, without alterations in hydrogen ion concentrations (i.e. pH). These studies revealed that wet nitrogen applications, in the form of ammonium nitrate, up to 18.5 $kg.ha^{-1}$ deposited (equivalent to 60 $kg.ha^{-1}.y^{-1}$ N) have little effect on plant biomass accumulation. Effects of exposures to SO_2 + NO_2 for the two species differed substantially from those found in Phase 1 of the programme when both species were unaffected by exposures up to and including 40 ppb SO_2 + 40 ppb NO_2. In this experiment, *B. media* showed transitional growth stimulation at low levels of SO_2 + NO_2 (not significant at the final harvest) while *A. capillaris* showed growth reductions at and above a threshold of 20 ppb SO_2 + 20 ppb NO_2. It is suggested that stage of plant development, time of year and temperature may influence the critical levels and loads of pollutants for individual species.

In conclusion, both positive and negative effects of pollutants on the growth of individual species may be expected to be critical in influencing the species composition of mixed swards. The total quantities and concentrations of pollutants which may cause these changes in growth vary substantially for different species and in relation to other environmental factors. The smallest applications of S + N found to stimulate growth in this programme were, for SO_2 + NO_2 gases, 0.32 $g.m^{-2}$ S + 0.10 $g.m^{-2}$ N (as 10 ppb SO_2 + 10 ppb NO_2 for 10 weeks) and, for wet deposition, 0.14 $g.m^{-2}$ S + 0.13 $g.m^{-2}$ N (applied as pH 4.5 mist over 18 weeks). The smallest applications of S + N found to reduce growth were, for SO_2 + NO_2 gases, 0.56 $g.m^{-2}$ S + 0.16 $g.m^{-2}$ N (as 20 ppb SO_2 + 20 ppb NO_2 for 11 weeks) and, for wet deposition, 0.77 $g.m^{-2}$ S + 0.66 $g.m^{-2}$ N (applied as pH 3.5 mist over 18 weeks).

2 INTRODUCTION

There has been much concern in the last decade because of the suggestion that 'acid rain' is causing extensive damage to vegetation in Europe and North America. Forest decline is considered to be indicative of the magnitude of the problem and the long-range transport of pollutants from industrial regions has been put forward as a possible cause (Innes, 1987). However, the evidence linking air pollution with vegetation damage is a matter of much controversy. Many of the symptoms reported to be indicative of pollution injury may also be induced by a variety of other factors. A further complication is that aerial pollutants are known to be capable of causing 'latent injury'. In such cases, damage occurs, but does not manifest itself until later stages of plant development.

There have been numerous studies, under controlled conditions of fumigation, which have aimed to assess the effects of gaseous pollutants on vegetation. Most studies have considered the effects of sulphur dioxide alone on plant growth, although some have considered the additional influences of nitrogen oxides and, to a lesser extent, ozone. A further limitation has been that most experiments have involved exposures to pollutants at concentrations much greater than those found in rural areas. Only in the recent years has emphasis moved away from studies of acute injury to plants, caused by high concentrations of phytotoxic gases, to investigations of the more subtle effects of long-term low-level exposures. In addition, the importance of wet deposition has been recognised as a major route for the influx of potentially damaging levels of sulphur, nitrogen and hydrogen ions into ecosystems. This is particularly evident in the uplands, where high levels of rainfall occur and vegetation may be cloaked for prolonged periods in highly acidic mists (Dollard, Unsworth & Harvey, 1983).

Surprisingly little research has been directed at studies of the effects of low levels of pollutants on semi-natural vegetation in the UK, particularly in relation to higher plants. However, Bell and his co-workers have provided evidence for the evolution of tolerance of grass species to SO_2 suggesting the past importance of this pollutant as an ecological factor in ecosystem responses (see Bell & Mudd, 1976; Bell, Rutter & Relton, 1979; Ashmore, Bell & Rutter, 1988). Further studies on ground flora in beech forests in Germany (Steubing *et al.*, 1986) have shown significant differences in species responses to intermitted fumigations with SO_2.

Long-term exposures of non-crop species to realistic concentrations of NO_x (under 100ppb) are poorly understood (Caporn, in press). Freer-Smith (1984) found 62ppb NO_2 to stimulate growth of Tilia cordata in the first year of treatment but the effect disappeared during the second year. Meanwhile, Wright (1987) found both a stimulation and a reduction in growth for different clones of *Betula pubescens* exposed to a weekly mean concentration of 62ppb NO_2. Research in our own laboratories has shown similar contrasting responses of different fern species to long-term fumigations with 60ppb NO_2 (Ashenden, Bell & Rafarel, 1990).

Extensive screening tests of native plant species to O_3 exposures have been carried out by Imperial College, London. These have shown some plant families (eg *Papilionoceae*) to contain a high proportion of sensitive species while others (eg *Compositae*) contain very few (Ashmore, 1984; Ashmore & Tickle, 1988). In general, species characteristic of calcareous habitats tend to be more sensitive than species characteristic of acid habitats. In longer-term exposures with rural concentrations, there have been several reports of growth reductions in response to O_3 eg. *Trifolium incarnatum* (Bennett & Runeckles, 1977) and hybrid poplars (Mooi, 1984). One of the most sensitive species reported is *Silene vulgaris* which showed a 63% reduction in dry weight and a total inhibition of flowering in response to 70 $\mu g\ m^{-3}$ O_3 for 12 hours per day for four weeks (Ernst *et al.*, 1985).

The responses of native plant species to combinations of the three major pollutants at low (= rural) concentrations has received even less attention. This is a particularly serious omission since, even in unpolluted atmospheres, O_3 occurs at concentrations of around 20ppb (Photochemical Oxidants Review Group, 1987). Research in the Netherlands (Mooi, 1984; Wolting & van Remortel, 1985) has shown fumigations with mixtures of NO_2, SO_2 and O_3 at 20-30ppb to cause marked reductions in the growth of *Plantago major* and *Populus nigra* whilst mixtures of NO_2 and O_3 or NO_2 and SO_2 caused only small growth reductions relative to charcoal filtered air controls. Further work by Ernst *et al.*, 1985 showed a mixture of 35ppb O_3 and 60ppb SO_2 for 4 weeks to caused reduced biomass and flowering in *Silene cucubalus* relative to plants exposed to 35ppb O_3 alone. Studies over a number of years by Mansfield and his co-workers at Lancaster have shown combinations of SO_2 and NO_2 to often be more toxic to plants than exposures to the pollutants applied alone (Ashenden & Mansfield, 1978; Whitmore & Freer-Smith, 1982; Whitmore, 1985; Wright *et al.*, 1986).

There have been numerous studies of the effects of wet deposition on crop species but results are conflicting especially at lower levels of acidity. Several authors have reported that simulated rain at high levels of acidity (pH below 3.0) may reduce primary production in plants (Harcourt & Farrar, 1980; Amthor, 1984). With less acid rain (viz.higher pH), most researchers have found little effect on plant responses. Indeed, in a review of the subject, Irving (1983) concluded that the effects on crops of ambient levels of rainfall acidity are likely to be minimal. However, Evans *et al.*, (1982, 1983) found that simulated acid rain at pH4 reduced the yields of field grown crops when compared to exposures at pH5.6 and it is known that plant sensitivity may differ during different stages of plant development (Caporn & Hutchinson, 1986).

Our own studies at ITE Bangor have suggested 9-170% yield reductions in winter barley grown on a range of British soils, in response to the normal ambient pH range of rainfall of 3.5 - 4.5 (Ashenden & Bell, 1987a). Subsequently, we reported both increased and decreased growths for different herbaceous and woody species exposed to the same rainfall treatments and have stressed the need to use large numbers of replicates in studies using native soils (Ashenden & Bell 1987b; 1988). Of the species tested, the most sensitive was Vicia faba L. which showed reductions in biomass for plants exposed to rain at pH4.5 as compared to ones supplied with rain at pH5.6 (Ashenden & Bell, 1989). More recently, we have reported enhanced growth of two upland plant species in response to highly acidic (pH2.5) fog/mist exposures. (Ashenden, Rafarel & Bell, 1991). These latest experiments have aimed to simulate events of occult precipitation, typical of upland habitats, and have involved much reduced deposits of treatment solutions over longer exposure times (6mm in 12h of misting as opposed to 24mm in about ½h of rain application). Thus, there have been smaller total inputs of sulphur, nitrogen and hydrogen ions for these experiments.

The majority of studies on the effects of wet deposition on plants have involved exposures to simulated rain/mist at different levels of acidity (hydrogen ion concentrations). In recent years it has become clear that the contribution of sulphur and nitrogen compounds is of great importance to the acid rain problem. Nitrogen, in particular is a major nutrient and its supply is often the growth limiting factor in natural ecosystems (Ellenberg, 1985). An increase in nitrogen supply initially stimulates biomass production but may cause a relative shortage of other nutrients like magnesium, potassium, phosphorus, molybdenum and boron (Nihlgard, 1985) and leads to an increased demand for other resources (Tilman, 1984). In addition, changes in nitrogen supply are known to play an important role in creation of new ecosystems during plant succession (Marrs *et al.*, 1983). Even small differences in species responses to nitrogen may lead to large differences in the botanical composition of plant communities (Bradshaw *et al.*, 1964; van Dijk & Roelofs, 1988). Continuous additions of nitrogen may eventually lead to ecosystems becoming saturated (Andersen, 1986; Roelofs, 1986). The implications of the continued large and increasing inputs of sulphur and nitrogen (as gases and in wet deposition) on the complex and diverse semi-natural plant communities of the UK are poorly understood. The research reported here aims to contribute to a National programme to assess the threat of these continuing atmospheric inputs by defining the critical loads for British vegetation types.

The critical loads concept was developed in the 1980's originally as a tool to help policy makers in decisions concerning reduction in anthropogenic emissions. By 1988, the concept was commonly accepted in the scientific community and a agreed definition developed by the UN-ECE Working Group on Nitrogen Oxides (Nilsson & Grennfelt, 1988): 'A quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge! This definition was later adopted at the UN-ECE workshop at Bad Harzburg (Anon.1990). The concept of critical loads now forms an important component of pollution control strategies in Europe (see review by Bull, 1991).

3 OBJECTIVES

This project forms part of an integrated research programme, involving also the Universities of Manchester and Newcastle-upon-Tyne, to determine the critical loads of deposited nitrogen and sulphur to natural and semi-natural ecosystems in the United Kingdom. The main thrust of the research at Bangor has been concerned with controlled environment studies. These studies have aimed to determine the effects of additions of atmospheric supplies of combined nitrogen and sulphur on the physiological and growth responses of a range of non-crop species from contrasting habitats. The research has followed three experimental phases:

Phase 1
The basic aim of Phase 1 was to determine whether the critical levels/loads of individual pollutants for native plant species are altered by the presence of other pollutant forms. The research was separated into three investigations.

a) An assessment of the separate and interactive effects of rural concentrations of ozone and acidic gases (ie sulphur dioxide and nitrogen dioxide) on plant growth.

b) An assessment of the effects of acidic mists (ie occult precipitation at different pHs) on plant growth. Critical loads of sulphur and nitrogen calculated for acidic inputs but with the confounding effects of large changes in hydrogen ion concentration.

c) An assessment of how the sensitivities of plant species to gaseous forms of pollutants may be affected by additional exposure to wet precipitation in the form of acidic mists.

Phase 2
The aim of Phase 2 was to determine dose-response relationships for a range of plants exposed to different concentrations of sulphur dioxide and nitrogen dioxide. This allows the determination of critical levels and, by calculation, an estimate of critical loads for sulphur and nitrogen in gaseous form.

Phase 3
The aim of Phase 3 was to determine the influence of additions of combined nitrogen (at the same pH) on dose-response relationships of selected species exposed to different concentrations of sulphur dioxide and nitrogen dioxide. This study removes any confounding effects of changes in hydrogen ion concentration in wet deposition.

4 EXPOSURE SYSTEMS

The pollution research facilities were designed at Bangor to allow studies of the effects on plants of both low, controlled concentrations of gaseous pollutants and acidic mists. The systems have been developed for studies involving shrubs or large numbers of potted plants.

Studies on the effects of gaseous pollutants on vegetation are conducted in four hemispherical glasshouses manufactured by Rosedale Engineering Ltd., of Filey, Yorkshire and marketed under the tradename 'Solardome 1'. The system is based on one originally designed in 1976 at the University of Lancaster (Ashenden *et al.*, 1982) and later modified (Lucas, Cottam & Mansfield, 1987) but the domes are smaller. Each dome has a diameter of 3.05 m, a floor surface area of 7.31 m^2 and is constructed of a glass mounted in an anodised aluminium framework (see Figure 1). The domes are ventilated by two 'Sonoxcarb' fan filter units (Machine Control Ltd., Horsham, Sussex). Each supplies two domes. Ambient air is drawn through charcoal filters, to remove any background levels of pollutants, and then pushed, via a flow–splitter, along the main air supply trunking to each dome. Next, the air enters a mixing chamber where controlled quantities of pollutant gases may be added before it is pushed into the dome and released around the circumference via a ring of lay–flat perforated polythene tube. Finally, the air leaves the system via a central chimney cowl at the top of the dome. The rate of air movement through each dome is adjustable up to 2 complete air changes per minute.

Gas control and delivery systems, together with gas analysers and a sampling system for monitoring dome gas levels, are housed in a nearby Systems Control building. All supply and sample lines are ducted underground to the domes. Ozone is generated by a laboratory ozonator (Wallace and Tiernan, Tonbridge, Kent). SO_2 and NO_2 are supplied from cylinders of compressed, pre–diluted gas as opposed to the pure gases used in the earlier, larger 'solardome' systems (see Lucas, Cottam & Mansfield, 1987). The advantage of this system is an easier control of pollutant concentrations without the need for expensive mass flow controllers. Instead, flows are maintained using constant differential regulation type controllers with needle valves (Rosemount Ltd., Stockport, Cheshire). All domes are fitted with supply lines for the three pollutants and PTFE sampling lines. Levels of pollutants in air sampled from the domes are measured using Monitor Labs. analysers for O_3 and NO_2 and a Dasibi analyser for SO_2. The fumigation facility and an assessment of operating performance has been fully reported elsewhere (Rafarel & Ashenden, 1991).

Studies on the effects of acid mists on plants are conducted in a closed 7.3 x 3.3 m polythene tunnel which is divided internally by polythene sheeting into four treatment bays. Each bay is fitted with a Sonicore atomising station (Lucas Dawe Ultrasonics, London) which can provide low volumes (0.4 – 8 $l.h^{-1}$) of treatment solutions at particle sizes which mostly lie in the 5–30μm range (equivalent to fogs/clouds). Treatment solutions are made up in the Systems Control building and pumped underground to the treatment bays where they are ultrasonically atomised in a compressed air stream. A full appraisal of the misting system has been reported elsewhere (Ashenden, Rafarel & Bell, 1991).

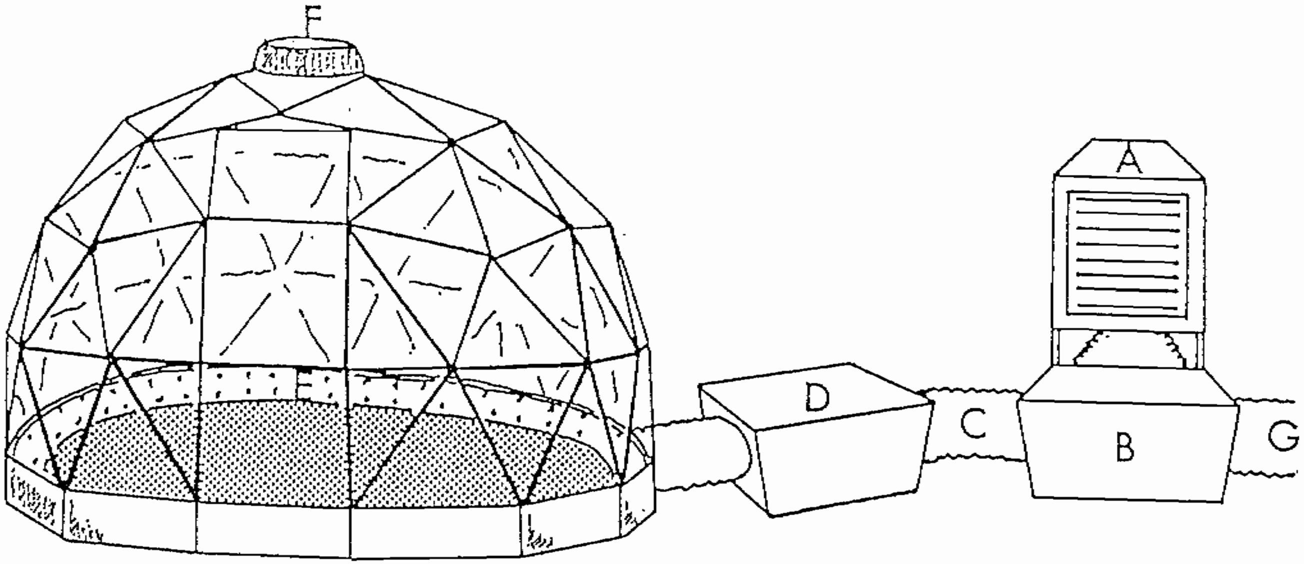

Figure 1. Diagram of 'solardome' fumigation chambers.
A, fan filter unit; B, flow splitter; C, air ducting;
D, mixing chamber; E, lay–flat tubing; F, ventilation cowl;
G, duct to second solardome chamber.

5 CRITICAL LOADS OF POLLUTANTS IN GASEOUS/WET ACID MIXTURES

5.1 Experimental treatments

The exposure facilities have been operated to supply the same series of pollution treatments throughout these experiments. All individual investigations have been fitted within this general scheme.

Atmospheres within the four 'Solardome' fumigation glasshouses were set to provide the following gaseous pollution treatments (a) charcoal filtered air (control), (b) SO_2 + NO_2, (c) O_3, (d) SO_2 + NO_2 + O_3. The concentrations of SO_2 and NO_2 were maintained at 40ppb (v/v) continuously. O_3 treatments were maintained at 40 ppb, except for two additional peaks per week of 1 x 3 h at 80 ppb on Thursdays and one of 3h at 80 ppb, immediately followed by 1h at 110 ppb O_3 on Thursdays. The peak concentrations for O_3 have been chosen to simulate those typically found in rural environments in the UK (see Photochemical Oxidants Review Group, 1987).

Mist solutions were made in deionised water by additions of equimolar concentrations of ammonium nitrate and sulphuric acid to provide four levels of acidity: (a) pH 2.5, (b) pH 3.5, (c) ph 4.5 and (d) pH 5.6. Mist treatments were applied in 3 x 4 h exposures (Mondays, Wednesdays and Fridays) each week to provide a total wet deposition of 6 mm per week. The length of misting and ionic balance of the mist solution was chosen so as to re-create conditions, in these respects, similar to those reported for Great Dun Fell (see Dollard, Unsworth and Harve, 1983). Supplementary watering of plants was provided by watering can at a rate of 24 mm per week using simulated acid rain solution of pH 4.5 (made up by additions of sulphuric and nitric acids in the ratio of 7:3, by volume). Hence the total quantity of rain and mist supplied was 30 mm per week.

A range of plant species have been exposed to the different gas and mist treatments. For studies involving exposure to combined gas and mist treatments, plants have been kept within the 'Solardome' fumigation system and transferred to the misting units only during the three misting events each week. For all other studies, plants have been kept within the respective exposure system continuously. The species studied, ages of plants and lengths of exposure to the treatments are shown in Table 1.

Table 1. Summary of the experimental exposures conducted

Species	Age of plants at start	Exposure Period (weeks)	Treatment
Lolium perenne	3 weeks from sowing	17	Mists
Holcus lanatus	3 weeks from sowing	13	Mists
Lotus corniculatus	4 weeks from sowing	18	Mists
Anthoxanthum odoratum	6 weeks from sowing	28	Mists
Holcus lanatus	3 weeks from sowing	15	Gases
Anthoxanthum odoratum	3 weeks from sowing	21	Gases
Nardus stricta	5 weeks from sowing	30	Gases
Lotus corniculatus	4 weeks from sowing	18	Gases
Deschampsia flexuosa	4 weeks from sowing	41	Gases
Trifolium repens	6 weeks from sowing	15	Mists x Gases
Lolium perenne	6 weeks from sowing	18	Mists x Gases
Agrostis capillaris	6 weeks from sowing	22	Mists x Gases
Juniperus communis	Cuttings	36	Mists x Gases

5.2 Results and Discussion

5.2.1 Mist Studies

The dry weight yields obtained for all four species exposed just to the four acid mist treatments are shown in tables 2 to 5. For all species, except *Lotus corniculatus*, there had been a greater accumulation of dry matter in the shoots and total plants in the most acid pH 2.5 treatment, as compared to all of the other less acid mist treatments. Generally, these higher dry weights at pH 2.5 were associated with increased root weights, but this was not significant ($P \leq 0.05$) for *Holcus lanatus*. For *Lolium perenne,* the greater dry weight accumulation in the pH 2.5 treatment was accompanied by an increased production of flowering stems ($P \leq 0.001$). The other species tested did not produce flowering stems during treatment exposures.

The most likely reason for the increased growth of plants in the most acid treatment is that the plants have been able to utilise the high levels of sulphur and nitrogen used in making up the mist solutions. It must be emphasised that at no time during the study did plants become pot–bound but the large inputs of total deposition (rain and mist) may have leached nutrients from the pots prior to the final harvest. The benefit to these species of the sulphur and nitrogen inputs via mist and rain solutions appear to have outweighed any adverse influences of the high hydrogen–ion concentration in the pH 2.5 mist.

Growth stimulation in response to wet acid deposition has been reported for some crop species (see Irving, 1983). Indeed our own investigations, on the effects of acidic rainfall on plants, have shown increased growth at pH 2.5 in some woody species and *Lolium perenne* (Ashenden & Bell, 1987b and 1988) but these have normally been accompanied by visible injury unlike in the present study. Our earlier studies used a much greater deposition of pH 2.5 treatment solution – 30 mm as opposed to 6 mm per week in the present series. While plants in the present work were supplied with a further 24 mm of pH 4.5 rain, this would not result in such large inputs of sulphur, nitrogen and, notably, hydrogen ions.

Plants of *Lotus corniculatus* responded differently to the exposure treatments, in comparison with the other species investigated. There was a greater dry weight of shoots and total plants in the pH 4.5 and pH 5.6 treatments as compared to the more acid pH 3.5 and pH 2.5 treatments. This higher dry matter accumulation was associated with greater root dry weights – significant at pH 4.5 ($P \leq 0.05$) but not quite significant at pH 5.6. It is interesting to note that plants exposed to the pH 4.5 treatment produced the greatest total dry weights and these were significantly higher ($P \leq 0.05$) than for plants exposed to the pH 5.6 (control) treatment. It is possible that small inputs of sulphur and nitrogen (as given in the pH 4.5 exposure) may stimulate growth, while higher levels (as in the pH 3.5 and pH 2.5 treatments), together with the increased hydrogen–ion input, may result in growth inhibition. In our earlier studies, we have reported growth inhibition in several species following exposure to simulated acid rain at pH 2.5 (Ashenden & Bell, 1987b). In this connection, it is important to note that *L. corniculatus* is a legume and these species were shown to be particularly sensitive to acid rain applications (Ashenden & Bell, 1989).

Table 2. **Mean numbers of flowering stems, root/shoot ratios and dry weights (g) of fractions of *Lolium perenne* (ryegrass) after being exposed to simulated mists at pHs of 2.5, 3.5, 4.5 and 5.6 for 17 weeks.**

	pH				L.S.D.
	2.5	**3.5**	**4.5**	**5.6**	
Dry weights					
Shoots	1.33	0.29	0.58	0.19	0.02
Roots	2.62	0.68	1.34	0.58	0.67
Total plant	3.95	0.97	1.92	0.77	0.80
Root/Shoot ratios	2.02	2.38	2.64	2.84	NS
No. flowering stems	0.93	0.07	0.00	0.00	0.67

L.S.D. = Least significant difference between treatments ($P \leq 0.05$)
NS = No significant differences between treatments

Table 3. **Mean dry weight yields (g) of fractions of *Holcus lanatus* L. (Yorkshire fog) and root/shoot ratios after being exposed to simulated mists at pHs of 2.5, 3.5, 4.5 and 5.6 for 13 weeks.**

	pH				L.S.D.
	2.5	3.5	4.5	5.6	
<u>Dry Weights</u>					
Shoots	3.90	2.58	2.12	2.43	0.46
Roots	2.35	2.47	2.08	2.02	NS
Total Plant	6.25	5.05	4.20	4.45	0.74
Root/Shoot ratios	0.60	0.99	0.99	0.88	0.15

L.S.D. = Least significant difference between treatments ($P \leq 0.05$)
NS = No significant differences between treatments

Table 4. **Mean dry weight yields (g) of fractions of *Lotus corniculatus* L. (Birdsfoot–trefoil) and root/shoot ratios after being exposed to simulated mists at pHs of 2.5, 3.5, 4.5 and 5.6 for 18 weeks.**

	pH				L.S.D.
	2.5	3.5	4.5	5.6	
Dry Weights					
Shoots	3.89	4.29	5.87	5.20	0.74
Roots	1.55	1.61	2.50	2.01	0.50
Total Plant	5.44	5.90	8.37	7.21	1.12
Root/Shoot ratios	0.40	0.38	0.42	0.38	NS

L.S.D. = Least significant difference between treatments ($P \leq 0.05$)
NS = No significant differences between treatments

Table 5. **Mean dry weight yields (g) of fractions of *Anthoxanthum odoratum* L. (sweet vernal-grass) and root/shoot ratios after being exposed to simulated mists at pHs of 2.5, 3.5, 4.5 and 5.6 for 28 weeks.**

	pH				L.S.D.
	2.5	3.5	4.5	5.6	
Dry Weights					
Shoots	7.39	3.28	3.67	2.93	0.84
Roots	3.75	2.73	3.21	2.42	0.55
Total Plant	11.14	6.01	6.88	5.35	1.29
Root/Shoot ratios	0.52	0.84	0.89	0.87	0.11

L.S.D. = Least significant difference between treatments (P ≤0.05)

There were different effects of the pH 2.5 treatment on the partitioning of assimilates. For *Holcus lanatus* and *Anthoxanthum odoratum*, there was a depressed root/shoot ratio which indicates a shift in the proportioning of assimilates away from the roots to the shoots. In contrast, there were no changes in root/shoot ratios with changes in plant productivity for the other two species. Differences in responses of species to wet deposition treatments are often reported in the literature (Irving, 1983; Evans, 1984) and effects on carbon partitioning need more investigation.

5.2.2 Gas studies

The five species exposed to the gaseous pollution treatments proved to be somewhat resistant to the pollutants. There were no significant differences ($P \leq 0.05$) between any treatments for dry weight yields of *Anthoxanthum odoratum* (Table 7), *Lotus corniculatus* (Table 9) or *Deschampsia flexuosa* (Table 10). For *Holcus lanatus*, there were no effects of treatments on shoot dry weights ($P \leq 0.05$) but there were effects on the final weights of roots (Table 6). There were higher weights of roots for plants of *Holcus lanatus* exposed to the O_3 and $SO_2 + NO_2$ treatments ($P \leq 0.05$) as compared to control plants and those exposed to the triple pollutant combination (ie. $SO_2 + NO_2 + O_3$ treatment). These higher yields of roots resulted in greater weights of total plants of *H. lanatus* for these treatments. The implication is that the low levels of $SO_2 + NO_2$ and O_3 used in these studies may stimulate growth in *H. lanatus*. This effect appears to be negated with the potentially more toxic combination of all three pollutants in the $SO_2 + NO_2 + O_3$ treatment.

The most notable effects of the gaseous pollution treatments were found for plants of *Nardus stricta*. For this species, plants grew biggest in the potentially most toxic, $SO_2 + NO_2 + O_3$ treatment (Table 8). There were significantly larger yields ($P \leq 0.05$) of shoots and total plants of *N. stricta* in the $SO_2 + NO_2 + O_3$ treatment in comparison to all other exposure treatments. In addition, there were larger root dry weights for plants exposed to O_3 and $SO_2 + NO_2 + O_3$ in comparison to controls and ones exposed to $SO_2 + NO_2$ ($P \leq 0.05$). The mechanism by which *N. stricta* is able to benefit from exposure to the triple pollutant combination is not clear from these studies but it is possible that this species has become adapted to growth in low levels of pollution exposure. It is a long-lived tussock grass which is frequently found in infertile, undisturbed sites and is particularly abundant in the uplands – regions now known to be subjected to relatively constant exposures of 25–40 ppb O_3 v/v throughout the year (Warren Spring, 1991).

5.2.3 Gas x mist studies

These studies revealed different responses and interaction effects of the gas x mist treatments on the growth of the various species studied.

An initial study on seedlings of *Trifolium repens* revealed significant differences ($P \leq 0.001$) between both the gaseous and acid mist treatments and a significant ($P \leq 0.001$) interactive effect of the treatments on total plant weights (see Table 11). All gas pollution treatments caused substantial yield reductions; averaged over all mist treatments, these were 16.6% for $SO_2 + NO_2$, 43.6% for O_3 and 59.4% for $SO_2 + NO_2 + O_3$ in comparison to control plants. For misting treatments, there was a lower yield averaged over all gas treatments at pH 2.5 compared to pH 4.5 and pH 5.6. The significant interaction of gas x mist treatments revealed

Table 6. **Dry weight yields (g) of fractions of *Holcus lanatus* L. (Yorkshire fog) after being exposed for 15 weeks to different air pollution treatments.**

	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	L.S.D.
Dry weights					
Roots	1.90	2.42	2.46	1.94	0.35
Shoots	2.66	3.14	3.03	2.36	NS
Total Plant	4.57	5.56	5.49	4.30	0.76
Root/Shoot Ratio	0.74	0.79	0.83	0.86	NS

L.S.D. = Least significant difference between treatments ($P \leq 0.05$)
NS = No significant differences between treatments

Table 7. **Dry weight yields (g) of fractions of *Anthoxanthum odoratum* L. (sweet vernal grass) after being exposed for 21 weeks to different air pollution treatments.**

	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control
<u>Dry weights</u>				
Roots	2.78	2.52	3.24	2.83
Shoots	3.91	3.41	3.72	3.87
Total Plant	6.69	5.93	6.96	6.70
Root/Shoot Ratio	0.73	0.77	0.87	0.74

There were no significant differences between treatments ($P \leq 0.05$)

Table 8. Dry weight yields (g) of fractions of *Nardus stricta* L. (mat-grass) after being exposed for 30 weeks to different air pollution treatments.

	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	L.S.D.
<u>Dry Weights</u>					
Roots	3.16	3.10	2.29	2.56	0.48
Shoots	4.81	3.42	3.62	3.09	0.78
Total Plant	7.97	6.52	5.91	5.65	1.18
Root/Shoot Ratio	0.68	0.92	0.67	0.83	0.10

L.S.D. = Least significant difference between treatments ($P \leq 0.05$)

Table 9. **Dry weight yields (g) of fractions of *Lotus corniculatus* L. (Birdsfoot–trefoil) after being exposed for 18 weeks to different air pollution treatments.**

	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control
Dry Weights				
Roots	1.64	1.75	1.84	1.85
Shoots	3.60	4.26	4.70	4.17
Total Plant	5.24	6.01	6.54	6.02
Root/Shoot Ratio	0.46	0.42	0.39	0.45

There were no significant changes between treatments ($P \leq 0.05$)

Table 10. **Dry weight yields (g) of fractions of *Deschampsia flexuosa (L) trin.* (wavy hair grass) after being exposed for 41 weeks to different air pollution treatments.**

	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control
Dry Weights				
Roots	1.61	0.93	1.32	1.15
Shoots	3.43	2.88	3.66	2.85
Total Plant	5.04	3.81	4.98	4.00
Root/Shoot Ratio	0.49	0.34	0.40	0.42

There were no significant differences between treatments ($P \leq 0.05$)

Table 11. **Dry weight yields (g) of fractions of *Trifolium repens* L. (white clover) after being exposed for 15 weeks to different aerial pollutants and acidified mist treatments.**

pH of mist treatment	Gas treatments				Mist treatment means
	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	
SHOOTS					
2.5	2.51	3.67	4.07	5.40	3.91
3.5	2.70	3.53	5.00	5.49	4.18
4.5	2.95	3.45	5.59	6.32	4.58
5.6	2.29	3.43	6.04	5.94	4.42
Gas treatment means	2.61	3.52	5.17	5.79	
L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.36					
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.72					
ROOTS					
2.5	0.51	0.82	0.99	1.69	1.00
3.5	0.46	0.75	1.29	1.62	1.03
4.5	0.56	0.88	1.19	2.19	1.20
5.6	0.43	0.70	1.34	1.87	1.09
Gas treatment means	0.49	0.79	1.20	1.84	
L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.14					
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.28					
TOTAL PLANTS					
2.5	3.02	4.49	5.06	7.09	4.92
3.5	3.16	4.28	6.29	7.12	5.21
4.5	3.50	4.33	6.78	8.51	5.78
5.6	2.72	4.13	7.37	7.82	5.51
Gas treatment means	3.10	4.31	6.38	7.64	
L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.44					
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.88					

that yield reductions attributed to the more acid mist treatments (pH 2.5 and pH 3.5) were not significant in O_3 and SO_2 + NO_2 + O_3 treatments; it seems that the severely harmful effects of these gaseous pollution treatments over-rode any effects of the mists. Generally, the effects of the treatments on the roots and shoots dry weight fractions were the same as those for total plants.

Similar effects of the gas treatments were found for seedlings of *Lolium perenne* (Table 12). Averaged over all mist treatments, there were reductions of 36.5% for O_3, 53.8% for SO_2 + NO_2 and 67.3% for SO_2 + NO_2 + O_3, in total plant weights, in comparison with control plants. The averaged effects of the mist treatments were the opposite to those found for *T. repens*; there was a greater average yield ($P \leq 0.001$) for plants exposed to the pH 2.5 mist as compared to the other mist treatments. However, a significant interaction effect of gases x mists was found and an examination of this revealed the higher yields for plants exposed to the pH 2.5 mist were only found ($P \leq 0.05$) for control and O_3 fumigated plants. It seems likely that the high levels of sulphur and nitrogen in the pH 2.5 mist are being utilised as plant nutrients in these gas treatments. Once again, the severely harmful effects of the SO_2 + NO_2 + O_3 treatment over-rode any influence of mist treatments. For plants in the SO_2 + NO_2 treatment, the highest yield was at pH 5.6 and this was significant when compared with plants exposed to the pH 2.5 misting treatment. A possible explanation for this observation is that these plants are already being harmed by sulphur- and nitrogen- containing gases (compare yield with control) and additional supplies of these elements via misting are increasing their toxicity.

The effects of the gas x mist treatments on seedlings of *Agrostis capillaris* are shown in Table 13. Once again, there were greater dry weights of green shoots for control plants as compared to the air pollution treatments ($P \leq 0.05$). However, observations on green shoot weights were not reflected in differences in total shoot weights where, as an average of all mist treatments, control plants were not significantly different ($P \leq 0.05$) from the rest. This is because of the lower weight of dead shoots recorded for control plants. For this species, there was a generally higher green shoot dry weight for plants exposed to the pH 2.5 mist treatment ($P \leq 0.05$) in comparison to the less acid mists, once again implicating a fertilizer effect.

Non-destructive measurements of growth for the one woody species (*Juniperus communis*) exposed to the gas x mist treatments are shown in Table 14. There were no significant effects of the mist treatments on the growth of rooted cuttings ($P \leq 0.05$) but exposure to the air pollution treatments resulted in reductions in shoot length after 36 weeks and these were greatest in the combined SO_2 + NO_2 + O_3 treatment ($P \leq 0.05$).

Table 12. Dry weight yields (g) of fractions of *Lolium perenne* L. (ryegrass) after being exposed for 18 weeks to different aerial pollutants and acidified mist treatments.

pH of mist treatment	Gas treatments				Mist treatment means
	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	
	G R E E N S H O O T S				
2.5	0.09	0.31	0.13	0.68	0.30
3.5	0.08	0.24	0.16	0.44	0.23
4.5	0.15	0.19	0.22	0.37	0.23
5.6	0.16	0.34	0.25	0.39	0.29
Gas treatment means	0.12	0.27	0.19	0.47	
	L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.06				
	L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.12				
	D E A D S H O O T S				
2.5	0.60	0.47	0.48	0.36	0.48
3.5	0.38	0.31	0.40	0.21	0.33
4.5	0.45	0.36	0.30	0.30	0.35
5.6	0.53	0.47	0.39	0.32	0.43
Gas treatment means	0.49	0.40	0.40	0.30	
	L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.06				
	L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.11				
	R O O T S				
2.5	1.31	5.31	1.18	7.53	3.83
3.5	0.90	1.97	1.87	4.59	2.33
4.5	0.92	1.50	1.77	2.08	1.57
5.6	1.20	1.62	2.47	3.56	2.21
Gas treatment means	1.08	2.59	1.82	4.43	
	L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.49				
	L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.97				
	T O T A L P L A N T S				
2.5	2.00	6.08	1.79	8.57	4.61
3.5	1.37	2.53	2.44	5.24	2.89
4.5	1.52	2.05	2.29	2.74	2.15
5.6	1.89	2.43	3.12	4.27	2.93
Gas treatment means	1.70	3.27	2.41	5.20	
	L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.52				
	L.S.D. ($P \leq 0.05$) between gas x mist treatments = 1.04				

Table 13. **Dry weight yields (g) of fractions of *Agrostis capillaris* L. (bent grass) after being exposed for 22 weeks to different aerial pollutants and acidified mist treatments.**

pH of mist treatment	Gas treatments $SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	Mist treatment means
		GREEN SHOOTS			
2.5	0.49	0.48	0.48	0.80	0.56
3.5	0.20	0.18	0.23	0.29	0.22
4.5	0.27	0.14	0.21	0.27	0.22
5.6	0.37	0.15	0.27	0.28	0.27
Gas treatment means	0.33	0.24	0.29	0.41	

L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.07
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.14

pH of mist treatment	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	Mist treatment means
		DEAD SHOOTS			
2.5	0.56	0.45	0.37	0.23	0.40
3.5	0.26	0.25	0.26	0.23	0.25
4.5	0.33	0.23	0.18	0.21	0.24
5.6	0.45	0.26	0.32	0.20	0.31
Gas treatment means	0.40	0.30	0.31	0.22	

L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.06
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.12

pH of mist treatment	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	Mist treatment means
		TOTAL SHOOTS			
2.5	1.05	0.94	0.84	1.03	0.97
3.5	0.46	0.43	0.48	0.52	0.47
4.5	0.60	0.37	0.39	0.48	0.46
5.6	0.82	0.41	0.58	0.48	0.57
Gas treatment means	0.73	0.54	0.58	0.63	

L.S.D. ($P \leq 0.05$) between gas or mist treatment means = 0.12
L.S.D. ($P \leq 0.05$) between gas x mist treatments = 0.23

Table 14. **Lengths of main shoots and total combined lengths of all shoots for plants of *Juniperus communis* L. after exposure for 24 and 36 weeks to different aerial pollutants and acidified mist treatments.**

pH of mist treatment	Gas treatments				Mist treatment means
	$SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	
After 24 Weeks					
	MAIN SHOOTS				
2.5	3.99	4.04	4.98	4.65	4.42
5.6	3.71	4.95	4.09	4.34	4.28
Gas treatment means	3.85	4.54	4.54	4.5	
	TOTAL SHOOTS				
2.5	10.79	13.38	17.20	13.66	13.76
5.6	10.49	16.16	10.56	13.48	12.67
Gas treatment means	10.64	14.77	13.88	13.57	
	There were no significant differences between treatments after 24 weeks				
After 36 weeks					
	MAIN SHOOTS				
2.5	4.91	5.40	6.44	6.99	5.94
5.6	4.93	6.79	5.64	6.85	6.05
Gas treatment means	4.92	6.09	6.04	6.92	
	L.S.D. ($P \leq 0.05$) between gas treatment means = 0.73 There were no significant effects of mist treatments				
	TOTAL SHOOTS				
2.5	15.33	22.44	34.93	44.65	29.34
5.6	15.88	27.89	21.02	44.96	27.44
Gas treatment means	15.61	25.17	27.97	44.80	
	L.S.D. ($P \leq 0.05$) between gas treatment means = 6.87 There were no significant effects of mist treatments				

5.3 Physiological measurements

Some physiological measurements were taken for plants of *Trifolium repens* (white clover) during the gas x mist exposure experiment. This species was chosen for further study because a) it was most sensitive to the exposure treatments and b) the broad leaves were easier to handle in the leaf chambers of the infra-red gas analyser. The object of these studies was to explore the potential for using physiological measurements as indicators of latent injury to plants.

Measurements of photosynthesis and stomatal conductance were taken using an ADC LCA2 portable infra-red gas analyser. The results are shown in Table 15.

While there was a tendency for lower rates of photosynthesis in plants exposed to the gas pollution treatments as compared to control plants, this was not significant ($P \leq 0.05$) because of a large amount of variation within treatments. However, there were significantly lower rates of photosynthesis ($P \leq 0.05$), as averaged effects over all gas exposures, for plants exposed to the more acid mists compared to the 'unpolluted' pH 5.6 mist treatment. This observation gives some support to the dry weights obtained (see Table 11).

For stomatal conductance, there were no differences between mist treatments (see Table 15) but lower rates for plants exposed to the gas pollution treatments, as compared to control plants. This indicates a tendency for stomatal closure in the presence of gaseous pollutants – an action suggesting the plants are under stress. It is important to point out that the measurements of photosynthesis and stomatal conductance were taken after removal of plants from the pollution treatments and represent only instantaneous measurements. They may not necessarily reflect long-term responses of the plants to the treatments.

Measurements were made of the rates of leaf drying for excised leaves taken from plants exposed to pH 2.5 and pH 5.6 mists for each gas exposure treatment. The leaves were removed by cutting the petiole near the base, cut petioles were coated in petroleum jelly to prevent water loss from the open wounds and then leaves were individually placed in open petri dishes on a laboratory bench. Each leaf was weighed within one minute of being excised and then at intervals up to 55 hours. The results are shown in Figures 2 and 3. It can be seen for each mist treatment that the rates of drying were least for plants which had been grown in charcoal filtered air (control) and greatest for ones which had been grown in the most damaging, $SO_2 + NO_2 + O_3$ treatment. An analysis of variance of the data after 55 hours showed a significantly greater rate of water loss from leaves of all gaseous pollution treatments as compared to control plants ($P \leq 0.05$) and confirmed that leaves from the plants in the $SO_2 + NO_2 + O_3$ treatment had suffered a greater rate of water loss than any other treatment ($P \leq 0.05$). In addition, there was a generally greater rate of water loss from plants exposed to the pH 2.5 as compared to the pH 5.6 mist ($P \leq 0.01$).

The leaf drying technique is considered to offer a measure of plant leaf responses to drought conditions and has been widely used (see Neighbour, Cottam & Mansfield,1988). The results obtained in the present study suggest that plants previously exposed to air pollution treatments and/or acid mists would be less capable of withstanding periods of drought. The noted reduced ability to retain leaf water may be because of a less effective control of stomatal closure or damaged cuticles which would result in greater rates of cuticular transpiration. Further investigations are required to determine the cause of increased water losses from the leaves of the polluted plants.

Table 15. **Mean rates of photosynthesis (μmol $CO_2.m^{-2}s^{-1}$) and stomatal conductance (μmol.$m^{-2}.s^{-1}$) for plants of *Trifolium repens* L. exposed to different aerial pollutants and acidified mist treatments.**

pH of mist treatment	Gas treatments $SO_2 + NO_2 + O_3$	O_3	$SO_2 + NO_2$	Control	Mist treatment means
		PHOTOSYNTHESIS			
2.5	5.44	6.18	4.94	6.02	5.65
3.5	5.13	7.04	6.30	6.91	6.34
4.5	6.00	7.26	6.57	7.19	6.75
5.6	7.27	7.63	7.28	8.60	7.69

L.S.D. ($P \leq 0.05$) between mist treatment means = 0.69
There were no significant differences between gas treatments

		STOMATAL CONDUCTANCE		
2.5	0.07	0.13	0.12	0.20
3.5	0.06	0.13	0.14	0.17
4.5	0.06	0.15	0.13	0.13
5.6	0.05	0.12	0.08	0.19
Gas treatment means	0.06	0.13	0.12	0.17

L.S.D. ($P \leq 0.05$) between gas treatment means = 0.03
There were no significant differences between mist treatments

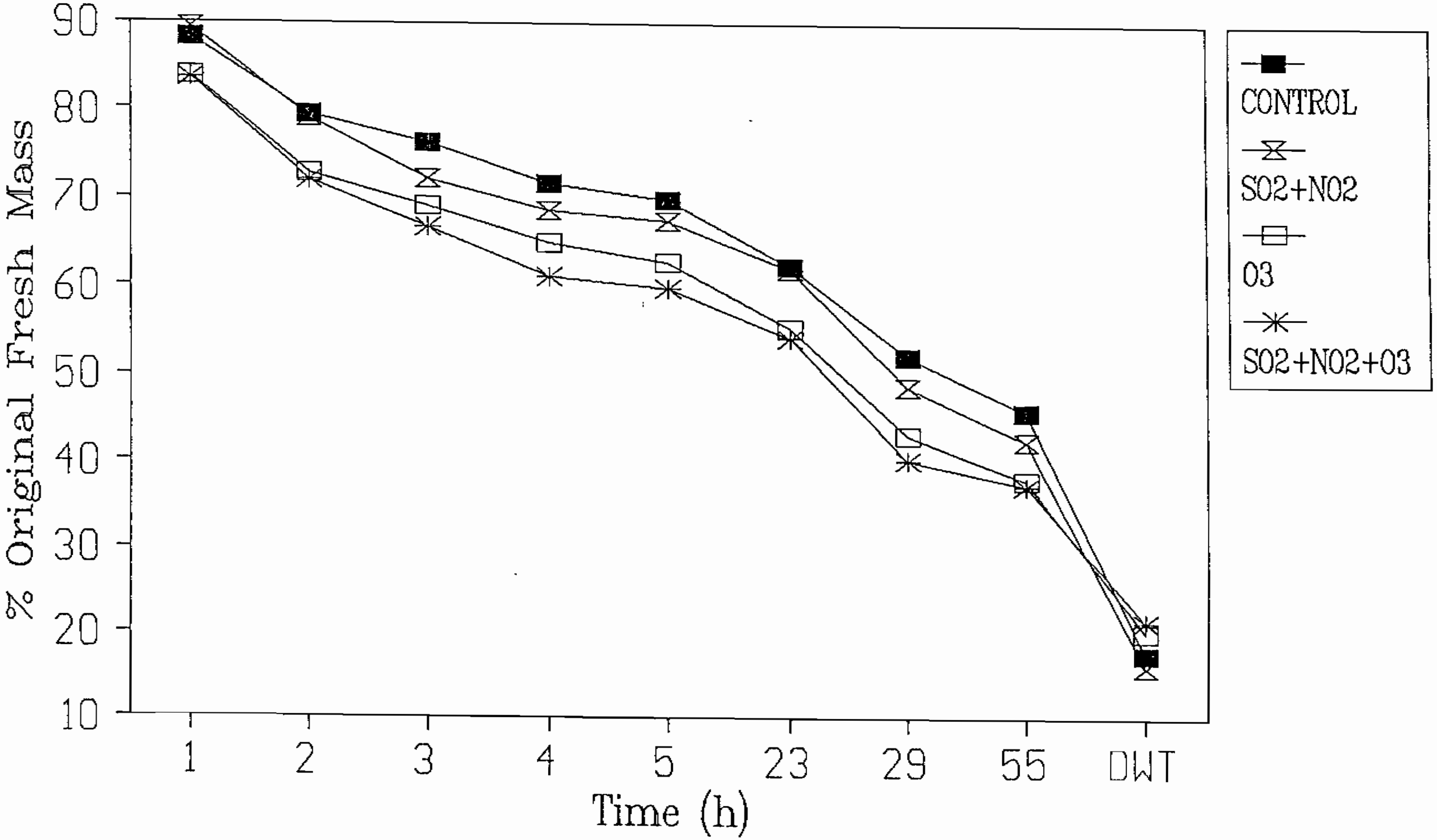

Figure 2. Alteration in fresh weight over time of leaves of *Trifolium repens* (white clover) after excision from plants previously exposed to different air pollution treatments and pH 5.6 mist.

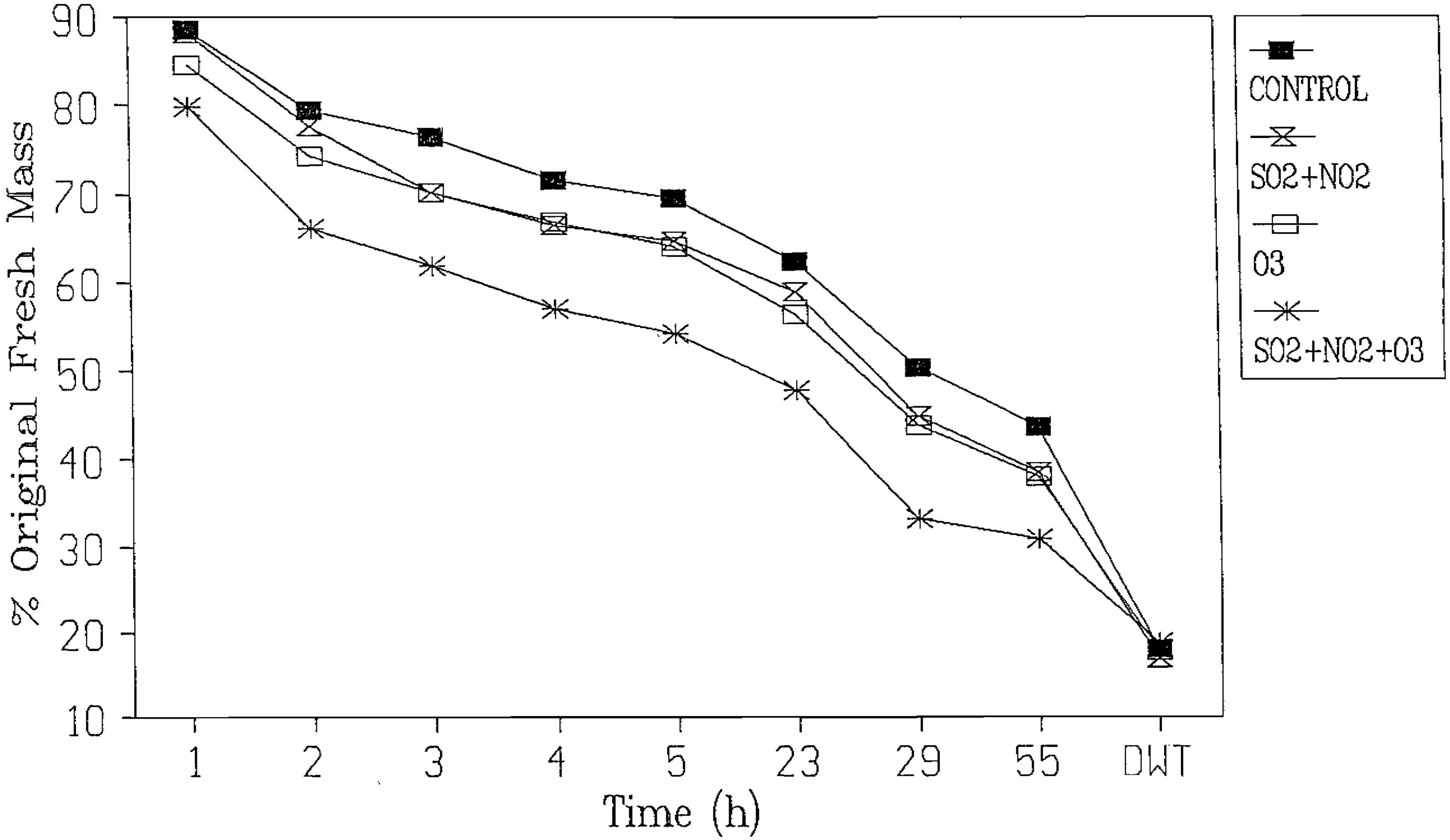

Figure 3. Alteration in fresh weight over time of leaves of *Trifolium repens* (white clover) after excision from plants previously exposed to different air pollution treatments and pH 2.5 mists.

5.4 Evaluation of critical loads

Total deposition rates for nitrogen and sulphur have been estimated for all mist, gas and mist x gas exposures in this phase of the programme (see Table 16). Estimates of wet deposition are based on calculations of the total sulphur and nitrogen supplied in treatment solutions and rates applied in the misting unit. Estimates of gaseous pollutant deposition have been supplied by Professor D Fowler and are based on computer models.

In the acid mist studies, three of the four species studied showed no differences in biomass accumulation for the pH 3.5, 4.5 and 5.6 treatments. This suggests that critical loads are above 0.62 $g.m^{-2}$ N and 0.72 $g.m^{-2}$ S for *Lolium perenne*, 0.48 $g.m^{-2}$ N and 0.55 $g.m^{-2}$ S for *Holcus lanatus* and 1.03 $g.m^{-2}$ N and 1.19 $g.m^{-2}$ S for *Anthoxanthum odoratum*. Positive growth responses for these three species were found in response to the pH 2.5 treatment but this supplied almost ten times the quantities of S and N in total deposition. Both positive and negative growth responses may be critical in terms of effecting changes in vegetation structure for mixed swards. However, the large differences in total S and N deposited for the pH 3.5 and pH 2.5 treatments makes a useful assessment of a critical load impossible.

Lotus corniculatus was most sensitive to the acid mist treatments. Plants exposed to the pH 3.5 and pH 2.5 treatments showed reduced biomass compared to those supplied with pH 4.5 and pH 5.6 mist. Thus the critical loads for adverse growth effects on this species will lie within 0.13 - 0.66 $g.m^{-2}$ N (1.3 - 6.6 $kg.ha^{-1}$) and 0.14 - 0.77 $g.m^{-2}$ S (1.4 - 7.7 $kg.ha^{-1}$). At the lowest end of this range (pH 4.5 mist @ 0.13 $g.m^{-2}$ N + 0.14 $g.m^{-2}$ S), there was a growth stimulation for *L. corniculatus*. Both stimulations and reductions in the growth of individual species may affect the composition of mixed swards and thus a deposition of S + N below this level may be regarded as critical. These levels are close to the range of wet deposition rates found for upland sites in Wales - 8–12 $kg.ha^{-1}$ y^{-1} S and 5–6 kg. ha^{-1} y^{-1} N (Donald & Stoner, 1989).

The five species exposed to gaseous SO_2 + NO_2 treatments alone proved to be generally resistant to the pollutants. Calculated deposition rates for these studies ranged between 1.13 to 1.92 $g.m^{-2}$ N and 2.11 to 4.56 $g.m^{-2}$ S. Interestingly, the only difference found in biomass production between control and SO_2 + NO_2 fumigated plants was for *Holcus lanatus* which was subjected to the lowest rate of total deposition (1.13 $g.m^{-2}$ N + 2.11 $g.m^{-2}$ S). This species showed increased root dry weights which were reflected in greater total plant weights. This stimulation in growth was reversed by the additional presence of O_3 indicating that any critical levels of SO_2 + NO_2 may be affected by the presence of other gaseous pollutants.

Studies on the effects of combinations of pollutant gases x acidic mists confirm that O_3 may influence the sensitivity of some species to SO_2 + NO_2. In addition, it is evident that wet and gaseous forms of sulphur and nitrogen deposition may have opposing effects on plant growth. Table 17 shows percentage changes in dry weights in relation to total sulphur and nitrogen deposition for plants grown in both charcoal filtered air (control) and 40 ppb SO_2 + 40 ppb NO_2 in combination with different acid mist treatments. For *Lolium perenne*, exposure to 7.01 $g.m^{-2}$ S + 6.12 $g.m^{-2}$ N as acid mist (pH 2.5) increases biomass two-fold whereas exposures to 2.00 $g.m^{-2}$ S + 0.81 $g.m^{-2}$ and above as SO_2 + NO_2 (with minimal wet inputs) significantly reduce biomass ($P \leq 0.05$). While both of these responses could dramatically affect species composition in mixed swards, it is apparent that critical loads and levels of S and N need to be identified separately for the different forms of pollutant deposition. For gaseous S + N, a critical load of below 2 $g.m^{-2}$ S + 0.81 $g.m^{-2}$ N is apparent.

Table 16. **Calculated deposition rates of sulphur and nitrogen in the separate experimental exposures to gases and mists (g.m^{-2}).**

Species	ACID MIST STUDIES							
	pH 2.5		pH 3.5		pH 4.5		pH 5.6	
	N	S	N	S	N	S	N	S
Lolium perenne	5.78	6.23	0.62	0.72	0.12	0.13	0.07	0.08
Holcus lanatus	4.42	5.07	0.48	0.55	0.09	0.10	0.06	0.06
Lotus corniculatus	6.12	7.01	0.66	0.77	0.13	0.14	0.08	0.09
Anthoxanthum odoratum	9.52	10.91	1.03	1.19	0.20	0.22	0.12	0.13

	Gas Studies (40 ppb SO_2 + 40 ppb NO_2)	
	N	S
Holcus lanatus	1.13	2.11
Anthoxanthum odoratum	1.32	2.70
Nardus stricta	1.85	3.74
Lotus corniculatus	1.29	2.40
Deschampsia flexuosa	1.92	4.56

Table 17. Percentage changes in total plant dry weights relative to plants grown in charcoal filtered air and exposed to a pH 5.6 mist (= 100%) and total rates of deposition of sulphur and nitrogen (g.m^{-2}) for plants exposed to the different gas x mist treatments.

pH of mist treatment	CONTROL			SO_2 & NO_2		
	% Total dry weight	Total S	Total N	% Total dry weight	Total S	Total N
			TRIFOLIUM REPENS			
5.6	100	0.07	0.06	94	2.05	1.01
4.5	109	0.12	0.11	87*	2.09	0.86
3.5	91	0.64	0.55	80*	2.61	1.50
2.5	91	5.84	5.10	65*	7.82	6.05
			LOLIUM PERENNE			
5.6	100	0.09	0.08	73*	2.00	0.81
4.5	64*	0.14	0.13	54*	2.06	0.86
3.5	123	0.77	0.66	57*	2.68	1.39
2.5	201*	7.01	6.12	42*	8.93	6.85
			AGROSTIS CAPILLARIS†			
5.6	100	0.11	0.09	121	2.21	0.81
4.5	100	0.17	0.16	81	2.28	0.87
3.5	108	0.94	0.81	100	3.04	1.52
2.5	215*	8.57	7.48	175*	10.68	8.19

* Indicates significant ($P < 0.05$) growth change with respect to control plants exposed to pH 5.6 mist.

† Shoot dry weights only

Similar differences in responses to gaseous and wet forms of S and N deposition occur for *Trifolium repens.* Here wet deposition up to and including 5.84 $g.m^{-2}$ S + 5.10 $g.m^{-2}$ N has no significant effects on plant growth ($P \leq 0.05$) while gaseous deposition of 2.09 $g.m^{-2}$ S + 0.86 $g.m^{-2}$ N (including 0.12 $g.m^{-2}$ S + 0.11 $g.m^{-2}$ N in mist) results in growth reductions. In contrast, for *Agrostis capillaris*, only high wet acid inputs affect biomass production and here growth is increased.

For all of the above investigations, wet applications of S and N are confounded by changes in acidity which may have direct influences on plant growth. Phase 3 of the experimental programme considers the effects of wet N deposition without alterations in hydrogen ion concentrations.

6 CRITICAL LOADS OF SO_2 + NO_2 POLLUTION

This investigation aimed to define dose–response relationships for a range of plants exposed to different concentrations of sulphur dioxide and nitrogen dioxide. Both sulphur dioxide and nitrogen dioxide are commonly found in the atmosphere together since they are both produced in the combustion of fossil fuels. The proportions of the two gases vary considerably from location to location and this is likely to affect plant responses. However, for this study the pollutants are fixed in equal proportions (by volume). The 10 species chosen are all represented in the field plots at Wardlow Haycop, under study in a related research programme by the University of Manchester.

6.1 Experimental Treatments

The 'Solardome' fumigation glasshouses were used for this investigation. They were set to provide treatments of (a) charcoal filtered air (control), (b) 10ppb SO_2+ 10ppb NO_2, (c) 20ppb SO_2 + 20ppb NO_2 and (d) 40ppb SO_2 + 40ppb NO_2.

Plants were raised from seed in trays of John Innes No.1 compost in mid January 1991. Approximately three weeks after sowing, seedlings were transferred to 0.5 litre pots containing a compost comprising 3 parts sterilised loam : 1 part fine grit with 1.54g. l^{-1} of Vitax Q4 fertiliser (Vitax Skelmersdale Lancs). The fertiliser addition was equivalent to one half of that used for a John Innes No.1 compost. Next 5 plants of each species were selected at random and placed in each of 3 experimental blocks in each of the Solardome glasshouses. Thus there were 15 plants of each species in each treatment.

Exposure treatments commenced on 15 February and were continued for 17 to 29 weeks before plants were removed from the Solardomes, washed free of soil and separated into roots and shoots for dry weight determinations. It was necessary to stagger harvest dates in order to handle the large amount of experimental material. The time of terminating experimental treatments for individual species depended on the sizes of the plants with larger plants being harvested first. All species were harvested prior to any possibility of becoming pot bound.

6.2 Results and Discussion

The data obtained are fully described in Tables 18–27. It is clear that there were substantial differences in the responses of the 10 species to the pollution treatments.

In six of the species - *Anthoxanthum odoratum* (Table 18), *Agrostis capillaris* (Table 19), *Hieracium pilosella* (Table 21), *Briza media* (Table 24), *Koeleria cristata* (Table 26) and *Deschampsia flexuosa* (Table 27) - there were no significant effects ($P \leq 0.05$) of the treatments on the dry weights of roots, shoots or total plants. The partitioning of assimilates, as expressed in root/shoot ratios, for these species were also unaffected except for *A. capillaris*. For *A. capillaris*, there was a higher root/shoot ratio for plants exposed to the 10ppb SO_2 + 10ppb NO_2 as compared to the other treatments ($P \leq 0.05$) which indicates a larger proportional diversion of assimilates to the roots. The general lack of growth responses in these six species suggests that the critical levels/loads for effects of the pollutants have not been exceeded in this investigation. It seems that higher concentrations of the pollutant combination are needed to exceed deposition rates above the critical loads for plant responses.

All of the remaining four species under investigation showed a stimulation in growth in response to at least one of the polluted air treatments in comparison to plants grown in charcoal-filtered air. For *Sanguisorba minor* (Table 20), *Galuim saxatile* (Table 22) and *Helictotrichon pratense* (Table 25), there was a higher dry weight of shoots at both the 10ppb SO_2 + 10ppb NO_2 and 20ppb SO_2 + 20ppb NO_2 treatments in comparison to plants exposed to 40ppb SO_2 + 40ppb NO_2 and charcoal filtered air ($P \leq 0.05$). This stimulation in growth with low levels of pollutants is likely to be a fertilizer effect whereby the plants are able to utilise the nitrogen (and possibly the sulphur) from the pollutant gases taken up by the plant. At the higher level of 40ppb SO_2 + 40ppb NO_2 the fluxes of pollutants into the leaves exceed levels at which they may be utilised for *S. minor* and *H. pratense,* where shoot weights at 40ppb SO_2 + 40ppb NO_2 are not significantly different from control plants ($P \leq 0.05$). For *G. saxatile*, there is a higher dry weight of shoots for plants in the 40ppb SO_2 + 40ppb NO_2 treatment than controls but growth stimulation is much less than for the lower pollutant levels.

The increased dry weights of shoots in the 10ppb SO_2 + 10ppb NO_2 and 20ppb SO_2 + 20ppb NO_2 treatments were associated with increased dry weights of roots ($P \leq 0.05$) for *G. saxatile* but not for *S. minor* and *H. pratense*. The stimulation in shoot growth for these treatments for all three species were reflected in significant differences ($P \leq 0.05$) in whole plant dry weights.

Plants of *Holcus lanatus* showed a higher dry weight of shoots in the 20ppb SO_2 + 20ppb NO_2 in comparison to all other treatments ($P \leq 0.05$). This was reflected in a higher dry weight of total plants ($P \leq 0.05$). There were no effects of treatments on root dry weights or root/shoot ratios for *H. lanatus*.

The contrasting responses of the different species to the air pollution treatments are difficult to explain. Effects are not related to length of exposure and thus total loads of pollutants deposited. Indeed, species exposed to both the shortest and longest periods show no growth responses. There is no relationship with 'Ellenberg' nitrogen valves or calcareous v acidic grassland species.

By definition the critical load of a pollutant (or combination of pollutants) is the quantity above which there is a harmful effect on a sensitive component of the ecosystem. In this study, no adverse effects of the pollutants were found for any species. However, positive growth responses as well as growth inhibition caused by pollutants may be expected to cause changes in vegetation structure if not all species in a community respond in the same manner. Hence, even the low levels of pollutants found to alter plant growth in these studies may be critical in terms of causing changes in vegetation patterns.

Table 18. **Mean dry weight yields (g) of fractions of *Anthoxanthum odoratum L.* (Sweet Vernal Grass) and root/shoot ratios after being exposed to a) Charcoal–filtered air, b) 10ppb SO_2 + 10ppb NO_2 c) 20ppb SO_2 + 20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 17 weeks.**

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	3.90	4.50	4.73	4.46	NS
Roots	2.34	2.47	2.61	2.85	NS
Total Plant	6.24	6.97	7.34	7.31	NS
Root/Shoot Ratios	0.61	0.57	0.57	0.67	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 19. Mean dry weight yields (g) of fractions of *Agrostis capillaris L.* (Common Bent-grass) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + NO_2, c) 20ppb SO_2 + 20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 19.5 weeks

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	4.73	4.44	5.29	4.47	NS
Roots	3.76	4.50	3.87	3.49	NS
Total Plant	8.49	8.94	9.16	7.96	NS
Root/Shoot Ratios	0.80	1.03	0.75	0.80	0.15

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 20. **Mean dry weight yields (g) of fractions of *Sanguisorba minor L.* (Salad Burnet) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 21 weeks**

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
<u>Dry Weights</u>					
Shoots	2.51	4.56	4.44	3.26	0.91
Roots	3.41	4.34	3.71	3.30	NS
Total Plant	5.92	8.90	8.15	6.56	1.44
Root/Shoot Ratios	1.39	1.03	0.87	1.06	0.21

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)

Table 21. **Mean dry weight yields (g) of fractions of *Hieracium pilosella L.* (Mouse–ear Hawkweed) and root/shoot ratios after being exposed to a) Charcoal–filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 22 weeks**

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	3.60	3.64	3.43	3.67	NS
Roots	1.54	1.80	1.60	1.34	NS
Total Plant	5.14	5.44	5.03	5.01	NS
Root/shoot Ratios	0.44	0.50	0.47	0.38	NS

L.S.D.= Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 22. **Mean dry weight yields (g) of fractions of *Galium saxatile L.* (Heath Bedstraw) and root/shoot ratios after being exposed to a) Charcoal–filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 23 weeks**

			ppb SO_2+ NO_2		L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	1.59	4.14	3.49	2.68	0.70
Roots	0.47	1.15	0.98	0.65	0.23
Total Plant	2.06	5.29	4.47	3.33	0.87
Root/Shoot Ratios	0.32	0.28	0.27	0.25	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 23. **Mean dry weight yields (g) of fractions of *Holcus lanatus L.* (Yorkshire Fog) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d)40ppb SO_2 + 40ppb NO_2 for 23.5 weeks.**

	ppb SO_2+ NO_2				L.S.D.
	0	10	20	40	
<u>Dry Weight</u>					
Shoots	2.12	2.35	2.62	2.03	0.40
Roots	1.70	1.72	2.01	1.70	NS
Total Plant	3.82	4.07	4.62	3.73	0.30
Root/Shoot Ratios	0.81	0.78	0.81	0.85	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 24. Mean dry weight yields (g) of fractions of *Briza Media L.* (Quaking Grass) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 26 weeks.

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
<u>Dry Weights</u>					
Shoots	2.68	2.84	2.76	3.05	NS
Roots	3.09	2.78	3.03	3.70	NS
Total Plants	5.77	5.62	5.79	6.75	NS
Root/Shoot Ratios	1.15	1.01	1.11	1.29	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 25. **Mean dry weight yields (g) of fractions of *Helictotrichon pratense L.* (Meadow Oat) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 28 weeks.**

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	1.61	2.47	2.34	1.96	0.64
Roots	0.98	1.53	1.55	1.24	NS
Total Plant	2.59	4.00	3.89	3.20	1.05
Root/Shoot Ratios	0.61	0.63	0.66	0.63	NS

L.S.D.= Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 26. **Mean dry weight yields (g) of fractions of *Koeleria cristata L.* (Crested Hair-grass) and root/shoot ratios after being exposed to a) Charcoal-filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 28.5 weeks.**

	ppb SO_2 + NO_2				L.S.D.
	0	10	20	40	
<u>Dry Weights</u>					
Shoots	2.78	2.54	3.06	2.74	NS
Roots	2.01	2.01	2.00	1.80	NS
Total Plant	4.79	4.55	5.06	4.54	NS
Root/Shoot Ratios	0.73	0.83	0.67	0.67	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

Table 27. **Mean dry weight yields (g) of fractions of *Deschampsia flexuosa L.* (Wavy Hair–grass) and root/shoot ratios after being exposed to a) Charcoal–filtered air, b) 10ppb SO_2 + 10ppb NO_2, c) 20ppb SO_2 +20ppb NO_2, d) 40ppb SO_2 + 40ppb NO_2 for 29 weeks**

			ppb SO_2 + NO_2		L.S.D.
	0	10	20	40	
Dry Weights					
Shoots	3.55	2.89	3.72	3.38	NS
Roots	1.34	1.23	1.41	1.29	NS
Total Plant	4.89	4.12	5.13	4.67	NS
Root/Shoot Ratios	0.39	0.43	0.38	0.39	NS

L.S.D. = Least significant difference between treatments ($p \leq 0.05$)
NS = No significant differences between treatments

6.3 Evaluation of Critical Loads

Estimates of total deposition rates of sulphur and nitrogen for the different SO_2 + NO_2 treatments and exposure periods have been derived from computer models by Professor D Fowler. These are shown for all species (see Table 28) together with corresponding changes in percentage total plant dry weights as compared to plants grown in charcoal filtered air (controls). It can be seen that there is no general pattern of plant growth responses in relation to total S and N deposition. Some species (e.g. *Deschampsia flexuosa*) show no alteration in biomass production up to loads of 3.64 $g.m^{-2}$ S + 1.78 $g.m^{-2}$ N (= 36.4 $kg.ha^{-1}$ S + 17.8 $kg.ha^{-1}$ N). Conversely, other species (e.g. *Sanguisorba minor*) show growth stimulation to inputs as low as 0.65 $g.m^{-2}$ S + 0.30 $g.m^{-2}$ N (6.5 $kg.ha^{-1}$ S + 3.0 $kg.ha^{-1}$ N). Plots of % biomass changes (all species) against SO_2 + NO_2 concentration (Figure 4), pollutant dose (Figure 5) reveal that it is not valid to plot a general response curve to describe the relationships with respect to all ten species (T Sparks pers. comm.). Plots for individual confirm growth stimulation to a maximum at 13 – 15 ppb SO_2 + NO_2 for *Sanguisorba minor, Galium saxatile, Holcus lanatus* and *Helictotrichon pratense* (see section 6.2).

Critical loads and levels are defined in terms of quantities of pollutants which affect sensitive elements of an ecosystem. Both growth stimulations and reductions in individual species may be expected to alter vegetation patterns. Hence, it is appropriate to consider a level of 10 ppb SO_2 + 10 ppb NO_2 and a total deposition of 6.5 $kg.ha^{-1}$ S + 3.0 $kg.ha^{-1}$ N to have possible influences on grasslands containing mixtures of species with different sensitivities.

Table 28. **Calculated deposition rates of sulphur and nitrogen in $SO_2 + NO_2$ dose-response studies ($g.m^{-2}$) and corresponding percentage changes in total dry weights of plants compared to controls (charcoal filtered air = 100%).**

Species	TREATMENT								
	10 ppb SO_2 + 10 ppb NO_2			20 ppb SO_2 + 20 ppb NO_2			40 ppb SO_2 + 40 ppb NO_2		
	% Dry weight	S	N	% Dry weight	S	N	% Dry weight	S	N
Anthoxanthum odoratum	112	0.51	0.23	118	1.02	0.47	117	2.04	0.93
Agrostis capillaris	105	0.60	0.28	108	1.20	0.56	94	2.41	1.12
Sanguisorba minor	150*	0.65	0.30	138*	1.29	0.61	111	2.59	1.21
Hieracium pilosella	106	0.68	0.32	98	1.36	0.64	97	2.73	1.28
Galium saxatile	257*	0.74	0.35	217*	1.47	0.70	162*	2.94	1.40
Holcus lanatus	107	0.75	0.36	121*	1.49	0.71	98	2.98	1.42
Briza media	97	0.82	0.39	100	1.64	0.79	117	3.27	1.58
Helictotrichon pratense	154*	0.89	0.43	150*	1.78	0.87	124	3.56	1.74
Koeleria cristata	95	0.90	0.44	106	1.80	0.88	95	3.61	1.76
Deschampsia flexuosa	84	0.91	0.45	105	1.82	0.89	96	3.64	1.78

* Indicates significant ($P \leq 0.05$) growth stimulation with respect to control plants

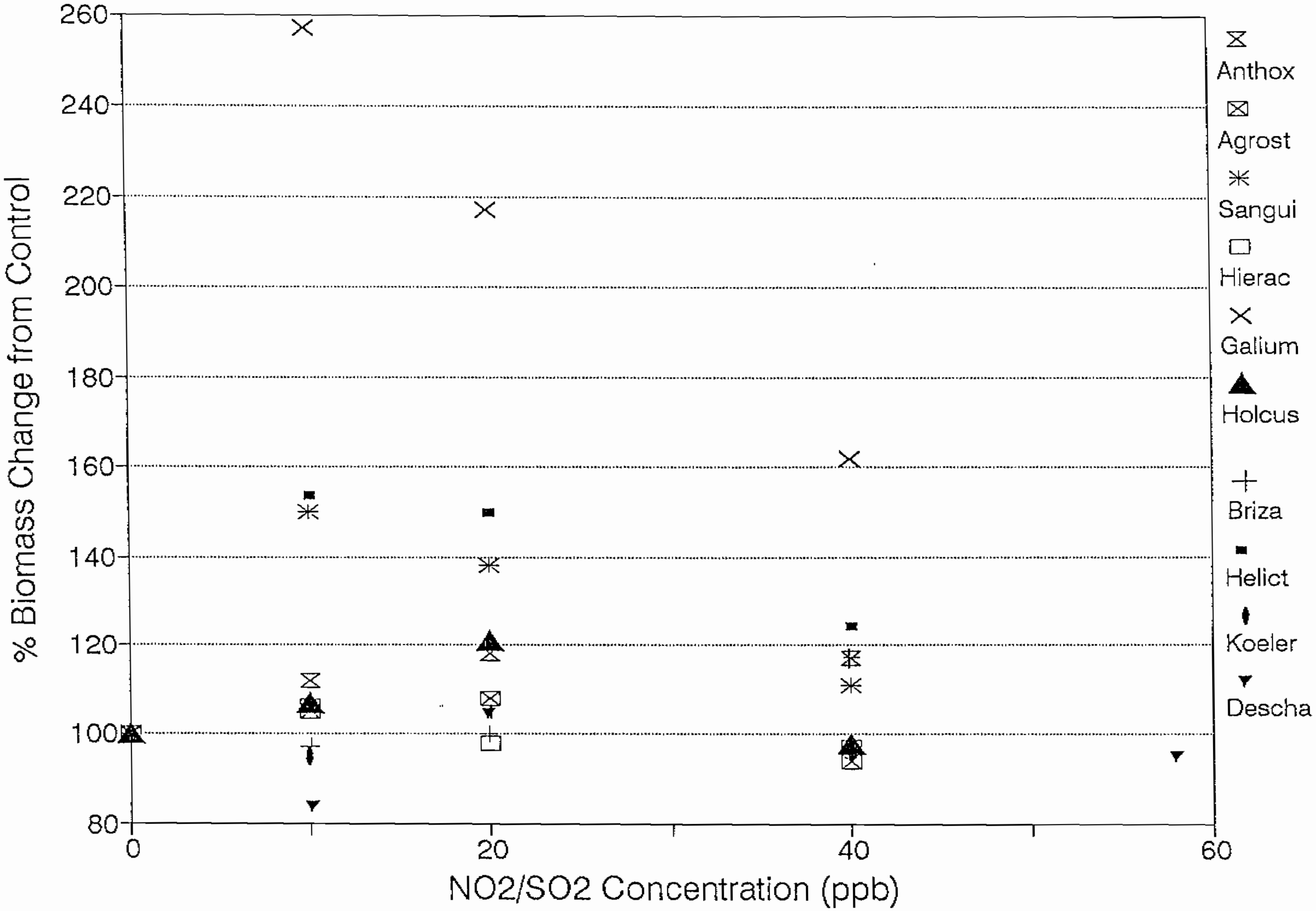

Figure 4. Plot of % dry weight (biomass) changes, with respect to growth in charcoal filtered air (control = 100%), against $SO_2 + NO_2$ concentration for a range of species.

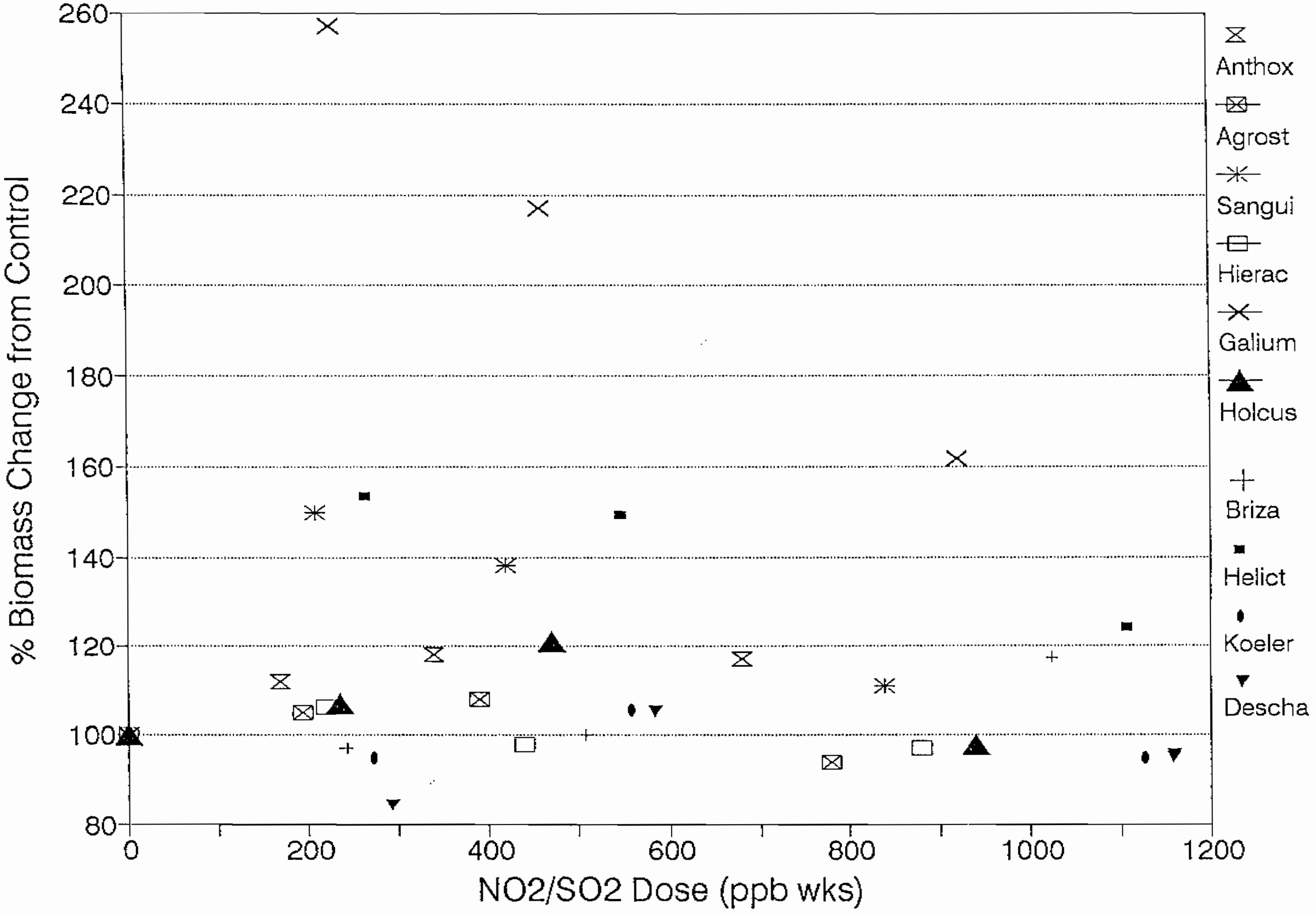

Figure 5. Plot of % dry weight (biomass) change, with respect to growth in charcoal filtered air (control = 100%), against dose (SO_2 + NO_2 x weeks) for a range of species.

7. CRITICAL LOADS OF GASEOUS SO_2 + NO_2 AND WET NITROGEN DEPOSITION

This phase of the programme aimed to determine if the critical levels/loads of gaseous pollutants are altered by additional inputs of wet nitrogen deposition. The earlier phases of the programme revealed contrasting critical levels/loads of SO_2 + NO_2 pollution for different native plant species (Chapter 6) and that responses to gaseous pollutants may be modified by additional inputs of wet acid deposition (Chapter 5). The mist treatments applied in the earlier work were based on different levels of acidity (pH) which confounds the influence of changes in hydrogen ion concentrations with the effects of wet sulphur and nitrogen additions on plant responses. The study reported here concentrates on changes in wet combined nitrogen additions without corresponding changes in sulphur or hydrogen ions. Responses are compared for a calcareous grassland species (*Briza media*) and an acidic grassland species (*Agrostis capillaris*) throughout a growing season.

7.1 Experimental Treatments

Seeds of *Agrostis capillaris* and *Briza media* were sown on 16 December 1991 in trays of John Innes No.1 compost. After 3 weeks, seedlings were transferred to 0.5 litre pots containing 3 parts sterilised loam : 1 part fine grit with $1.54 g.l^{-1}$ Vitax Q4 fertilizer (Vitax Ltd., Skelmersdale, Lancs). The fertilizer addition was one half of that normally used for a John Innes No.1 potting compost. The following day, 27 pots of each species were placed in each of 4 blocks within each of the four Solardome glasshouses and left to become established and acclimatise to the environmental conditions. Experimental treatments commenced on 24 January.

The Solardome glasshouses were set to provide treatments of (a) charcoal filtered air (b) 10ppb SO_2 + 10ppb NO_2 (c) 20ppb SO_2 + 20ppb NO_2 (d) 40ppb SO_2 + 40ppb NO_2. The four treatment blocks within each Solardome were randomly allocated different wet deposition treatments. These treatments were applied as fine mists using an updated garden sprayer with a compressed air supply to ensure uniform delivery across blocks of plants. The treatment solutions were designed to provide a 'North Wales maritime rain' with the equivalent of 0, 20, 40 or 60 Kg N per hectare per year. The composition of the rain solution was based on analyses of precipitation collected between 1984 and 1986 in North Wales (Reynolds, Williams & Stevens, 1990) with nitrogen levels adjusted by additions of ammonium nitrate. Simulated rain was applied 5 days per week to give a total deposition equivalent to 2500mm per year. Supplementary watering with de-ionised water was applied at weekends by watering can.

Nine plants from each gas x rain treatment were taken from the Solardomes at 11, 13 and 15 weeks for *Agrostis capillaris* and at 12, 14 and 16 weeks for *Briza media*, after exposures commenced. On each occasion, plants were washed free of soil, numbers of tillers and leaf areas were recorded and plants separated into leaves, stems and roots for dry weight determinations.

The data obtained were interpreted by analysis of variance techniques using gases (levels of SO_2 + NO_2) and nitrogen treatments (wet deposition) as main effects.

7.2 Results and Discussion

There were contrasting effects of the air pollution treatments on the growth of the two species (see Figures 6 and 7). Overall, for *Agrostis capillaris*, there was a reduction in plant growth at both the 20ppb SO_2 + 20ppb NO_2 and the 40ppb SO_2 + 40ppb NO_2 treatments compared to the plants exposed to charcoal filtered air and the 10ppb SO_2 + 10ppb NO_2 treatment. In contrast, for *Briza media*, there was a trend for increased growth for plants exposed to the 10ppb SO_2 + 10ppb NO_2 and 20ppb SO_2 + 20ppb NO_2 treatments as compared to controls (charcoal filtered air) and plants exposed to the most polluted 40ppb SO_2 + 40ppb NO_2 treatment. However, this was not quite significant ($p \leq 0.05$) at the final harvest. Generally, there were no overall effects of the different wet nitrogen applications on plant growth. The only exception was a lower root dry weight for all nitrogen additions above the control (zero application) for *Agrostis capillaris* at the final harvest.

The changes in total plant dry weights for both species were generally reflected in significant ($P \leq 0.05$) changes in dry weights of the separate plant fractions (roots, stems and leaves). There were no effects of the treatments on partitioning of assimilates for *Briza media* ($P \leq 0.05$). However, for *Agrostis capillaris*, there was a tendency for increased root/shoot ratios with increasing levels of SO_2 + NO_2 pollution ($P \leq 0.05$). This indicates that growth reductions in the higher air pollution treatments were a result of a disproportionate (more adverse) effect on the growth of aerial plant fractions as compared to roots (see Figure 7).

Changes in plant dry weights were not associated with differences in the numbers of tillers produced for *Briza media* except at the initial harvest (Table 29). However, for *Agrostis capillaris*, there was a transitional effect of air pollution treatments on numbers of tillers after 13 weeks. Lower numbers of tillers were recorded at the higher levels of SO_2 + NO_2 for this species (Table 30). There was also an overall reduction in tillers for plants of *Agrostis capillaris* at the highest wet nitrogen application (equivalent of 60 $Kg.N.ha^{-1}.y^{-1}$) compared to other treatments at the final harvest.

There was a general trend for changes in leaf area in association with the alterations in dry weight accumulation for both species. For *Briza media*, there were larger leaf areas at the 10ppb SO_2 + 10ppb NO_2 and 20ppb SO_2 + 20ppb NO_2 as compared to other treatments (Table 31). Meanwhile for *Agrostis capillaris*, the lower dry weights at the 40ppb SO_2 + 40ppb NO_2 treatment were associated with an overall lower leaf area (Table 32).

By definition, the critical load of a pollutant (or combination of pollutants) is the quantity above which there is a harmful effect on a sensitive component of the ecosystem. In this study, both enhanced and reduced growth were found in response to the pollution treatments for the different species. Both responses may equally influence long-term changes in vegetation patterns. These possible changes will require further evaluation in future studies on mixed species swards.

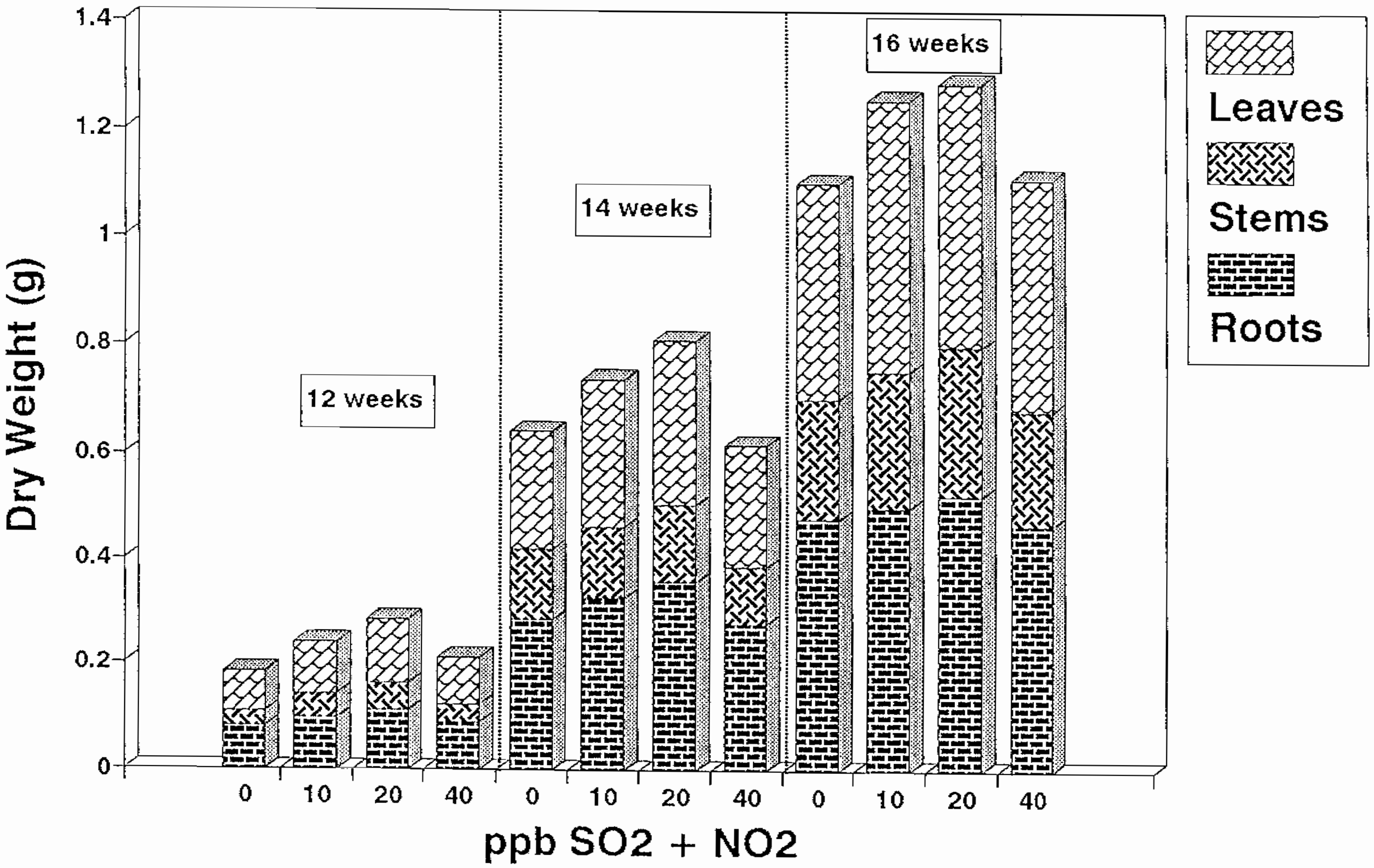

Figure 6. Dry weights of fractions of *Briza media* after being exposed to different levels of SO_2 and NO_2.

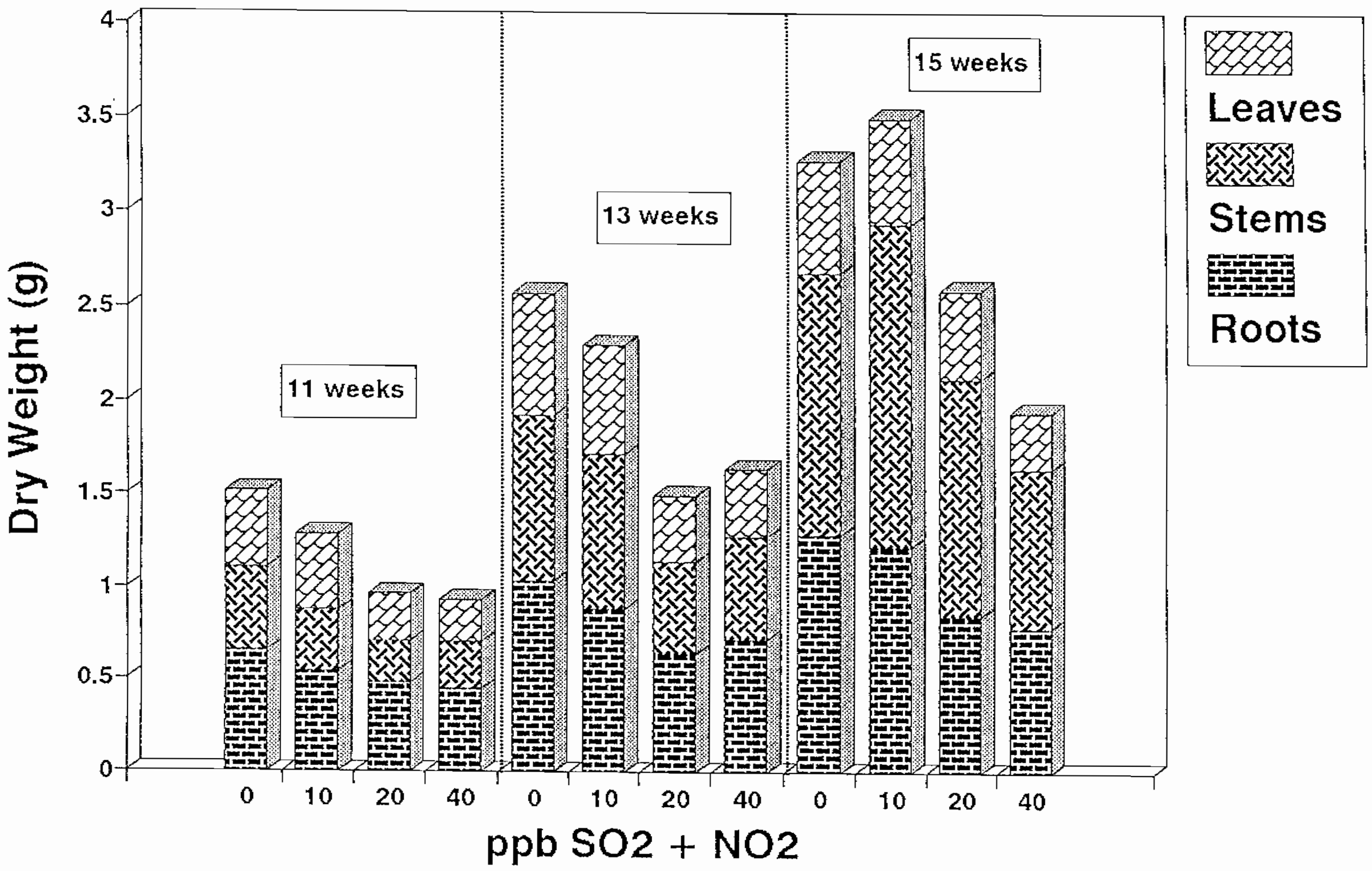

Figure 7. Dry weight fractions of *Agrostis capillaris* after being exposed to different levels of SO_2 and NO_2.
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Table 29. Numbers of tiller of *Briza media* after being exposed for 12, 14 and 16 weeks to different levels of $SO_2 + NO_2$ and wet nitrogen applications.

	ppb $SO_2 + NO_2$				
Nitrogen additions $Kg.ha.^{-1}.y^{-1}$	0	10	20	40	Nitrogen treatment means
AFTER 12 WEEKS					
0	5.11	6.22	6.78	5.78	5.97
20	5.44	6.33	6.44	5.00	5.80
40	4.22	6.67	5.44	6.11	5.61
60	5.00	5.89	6.67	4.78	5.58
Gas treatment means	4.94	6.28	6.33	5.42	5.74

L.S.D. ($p \leq 0.05$) between gas treatments = 0.90
There were no significant differences
between nitrogen treatments ($p \leq 0.05$)

AFTER 14 WEEKS					
0	6.67	8.78	7.78	8.44	7.92
20	6.78	8.89	8.22	8.78	8.17
40	7.33	8.44	9.44	8.44	8.42
60	7.89	6.67	9.00	7.44	7.75
Gas treatment means	7.17	8.19	8.61	8.28	8.06

There were no significant differences
between gas or nitrogen treatments ($p \leq 0.05$)

AFTER 16 WEEKS					
0	9.00	10.89	8.89	9.56	9.58
20	10.33	9.56	8.89	9.33	9.53
40	7.67	10.89	11.56	10.33	10.11
60	10.44	9.67	13.33	11.89	11.33
Gas treatment means	9.36	10.25	10.67	10.28	10.14

There were no significant differences
between gas or nitrogen treatments ($p \leq 0.05$)

Table 30. Numbers of tillers of *Agrostis capillaris* after being exposed for 11, 13 and 15 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb $SO_2 + NO_2$				
Nitrogen additions $Kg.ha^{-1}.y^{-1}$	0	10	20	40	Nitrogen treatment means
AFTER 11 WEEKS					
0	35.78	24.67	31.67	26.33	29.61
20	32.78	28.78	38.78	26.89	31.81
40	29.78	29.67	29.67	23.44	18.14
60	32.11	29.78	29.11	25.22	29.06
Gas treatment means	32.61	28.22	32.31	25.47	29.65
There were no significant differences between gas or nitrogen treatments ($p \leq 0.05$)					
AFTER 13 WEEKS					
0	50.89	61.89	33.22	58.78	51.19
20	42.00	54.33	28.67	23.22	37.06
40	40.22	50.22	42.00	33.78	41.56
60	48.67	36.44	35.78	43.67	41.14
Gas treatments means	45.44	50.72	34.92	39.86	42.74
L.S.D. ($p \leq 0.05$) between gas treatments and nitrogen treatments = 8.69					
AFTER 15 WEEKS					
0	42.00	52.56	45.44	51.67	47.92
20	39.44	39.89	38.78	42.78	40.22
40	41.11	50.56	37.44	41.56	42.67
60	28.44	33.11	37.44	37.33	34.08
Gas treatments means	37.75	44.03	39.78	43.33	41.22

L.S.D. ($p \leq 0.05$) between nitrogen treatments = 8.16
There were no significant differences
between gas treatments ($p \leq 0.05$)

Table 31. Leaf areas of *Briza media* after being exposed for 12, 14 and 16 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
AFTER 12 WEEKS					
0	19.17	20.30	20.45	22.27	20.55
20	15.97	16.28	19.40	17.23	17.22
40	15.56	26.54	15.19	22.31	19.90
60	25.28	19.49	24.87	12.03	20.42
Gas treatment means	18.99	20.65	19.98	18.46	19.52

There were no significant differences between gas or nitrogen treatments ($p \leq 0.05$)

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
AFTER 14 WEEKS					
0	39.59	92.43	71.73	43.09	61.71
20	48.52	51.80	68.92	63.46	58.17
40	52.94	82.89	78.88	56.17	67.72
60	56.64	50.68	78.64	44.95	57.73
Gas treatment means	49.92	69.45	74.54	51.92	61.33

L.S.D. ($p \leq 0.05$) between gas treatments = 9.97
There were no significant differences between nitrogen treatments ($p \leq 0.05$)

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
AFTER 16 WEEKS					
0	98.01	126.26	95.79	96.00	104.01
20	89.23	112.74	105.73	102.51	102.55
40	78.25	134.88	124.90	106.09	111.03
60	126.14	116.83	163.42	88.11	123.63
Gas treatment means	97.91	122.68	122.46	98.18	110.31

L.S.D.($p \leq 0.05$) between gas or nitrogen treatments = 14.14

Table 32. Leaf areas of *Agrostis capillaris* after being exposed for 11, 13 and 15 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
		AFTER 11 WEEKS			
0	90.70	114.44	69.59	91.55	91.57
20	118.64	104.94	69.68	86.94	95.05
40	78.19	121.48	76.99	58.35	83.75
60	118.03	111.01	79.71	53.53	90.57
Gas treatment means	101.39	112.97	73.99	72.59	90.23

L.S.D. ($p \leq 0.05$) between gas treatments = 21.46
There were no significant differences
between nitrogen treatments ($p \leq 0.05$)

AFTER 13 WEEKS

0	229.67	271.08	134.48	252.79	222.01
20	250.02	176.15	160.29	134.69	180.29
40	177.04	228.02	105.44	87.16	149.41
60	262.28	149.38	159.09	137.01	176.94
Gas treatments	229.75	206.16	139.82	152.91	182.16

L.S.D. ($p \leq 0.05$) between gas treatments
or nitrogen treatments = 31.66

AFTER 15 WEEKS

0	282.92	211.66	201.39	145.52	210.37
20	168.52	190.63	198.78	101.98	164.98
40	137.67	255.70	205.89	100.87	175.03
60	188.29	241.95	180.18	132.42	185.71
Gas treatment means	194.35	224.99	196.56	120.20	184.02

L.S.D. ($p \leq 0.05$) between gas treatments
or nitrogen treatments = 32.01

7.3 Physiological measurements

The physiological responses of both *Agrostis capillaris* and *Briza media* to the different air pollution x wet nitrogen treatments were monitored throughout this investigation. Just prior to each destructive harvest (except harvest 1 for *B. media*), plants were taken from each experimental treatment and photosynthetic rates determined for the largest fully expanded leaf using an ADC LCA2 portable infra-red gas analyser. Leaves of *B. media* were not measured prior to the first harvest because they were too small to position within the leaf monitoring chambers. Further measurements of stomatal conductance were taken within treatment domes using a Delta-T AP4 porometer prior to each harvest.

Tables 33 and 34 show mean rates of photosynthesis for plans of *B. media* and *A. capillaris* respectively. For both species, there were transitional effects of the treatments on photosynthetic rate which had disappeared by the final harvest. In the case of *B. media*, there were increased rates of photosynthesis in response to both the 10 ppb SO_2 + 10 ppb NO_2 and 20 ppb SO_2 + 20 ppb NO_2 treatments at 13 weeks. This observation reflects the increased dry matter production for *B. media* ($P \leq 0.05$) at these two gas exposures. In addition, there were increased rates of photosynthesis at 20 and 40 $kg.ha^{-1}$ N applications as compared to the extreme 0 and 60 $kg.ha^{-1}$ N treatments. These changes in photosynthetic rate in response to N were not reflected in changes in dry matter production for *B. media* and are regarded as short-term responses with no long-term influence on growth. Alternatively, there may be a general increase in metabolic rate in response to intermediate N applications whereby respiration is increased as much as photosynthesis.

For *A. capillaris*, the only effect of treatments on rates of photosynthesis occurred after 10 weeks where plants exposed to applications of 40 $kg.ha^{-1}$ N showed reductions compared to other nitrogen applications ($P \leq 0.05$). In contrast to observations on dry matter production, there were no effects of gas exposure treatments on photosynthetic rate for this species. Measurements of net photosynthesis are taken instantaneously and it is possible that transitional effects of the gas treatments may have occurred at earlier growth stages.

The effects of treatments on stomatal conductance are shown in Tables 35 (*B. media*) and 36 (*A. capillaris*). Again, there were transitional effects of the treatments which disappeared following the first measurement period. For *B. media*, there was a reduction in stomatal conductance for the abaxial leaf surface in response to the two highest (20 and 40 ppb) SO_2 + NO_2 treatments ($P \leq 0.05$). This may indicate partial stomatal closure in order to reduce pollutant uptake. However, there were no effects of treatments on stomatal conductance for the more photosynthetically active adaxial leaf surface. In contrast, for *A. capillaris*, there were no effects of gas treatments on stomatal conductance.

7.4 Evaluation of critical loads

The wet deposition treatments of up to and including a rate of application of 60 $kg.ha^{-1}.y^{-1}$ N had no overall effects on biomass production for *Briza media* and *Agrostis capillaris*. Actual quantities deposited were 1.85 $g.m^{-2}$ N (18.5 $kg.ha^{-1}$ N) after 16 weeks for *B. media* and 1.73 $g.m^{-2}$ N (17.3 $kg.ha^{-1}$ N) after 15 weeks for *A. capillaris*. It is apparent that these quantities are below the critical loads for wet deposition for the two species.

Table 33. Mean rates of photosynthesis (μmol $CO_2.m^{-2}$ s^{-1}) for plants of *Briza media* after being exposed for 13 and 15 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
		AFTER 13 WEEKS			
0	8.41	10.59	10.05	7.58	9.16
20	9.64	14.77	18.55	14.50	14.37
40	10.98	16.92	11.03	13.88	13.20
60	8.95	9.74	12.67	8.64	10.00
Gas treatment means	9.49	13.01	13.08	11.15	

L.S.D. ($p \leq 0.05$) between gas treatments and nitrogen treatments = 2.79

		AFTER 15 WEEKS			
0	3.43	7.13	3.75	4.57	4.72
20	4.03	5.53	4.35	4.68	4.65
40	6.03	6.76	6.04	5.68	6.13
60	5.47	4.41	4.78	4.58	4.81
Gas treatment means	4.74	5.96	4.73	4.88	

There were no significant differences between gas or nitrogen treatments ($P \leq 0.05$)

Table 34. Mean rates of photosynthesis (μmol $CO_2.m^{-1}.S^{-1}$) for plants of *Agrostis capillaris* after being exposed for 10, 12 and 14 weeks to different levels of SO_2 and NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions $Kg.ha^{-1}.y^{-1}$	0	10	20	40	Nitrogen treatment means
		AFTER 10 WEEKS			
0	14.36	19.20	18.06	15.51	16.78
20	15.13	16.99	16.56	17.70	16.44
40	13.65	11.97	12.57	10.99	12.30
60	15.07	17.16	13.86	14.80	15.22
Gas treatment means	14.55	16.33	15.26	14.59	

L.S.D. ($p \leq 0.05$) between nitrogen treatments = 2.57
There were no significant
differences between gas treatments

AFTER 12 WEEKS

0	18.33	16.38	21.20	14.75	17.67
20	17.17	16.45	19.13	20.62	18.34
40	12.58	15.55	18.90	17.29	16.08
60	11.54	14.05	18.30	15.71	14.90
Gas treatment means	14.91	15.61	19.38	17.09	

There was no significant differences between gas
or nitrogen treatments ($P \leq 0.05$)

AFTER 14 WEEKS

0	27.74	21.54	14.89	19.23	20.85
20	25.87	19.66	34.71	22.36	25.65
40	17.45	17.74	22.00	24.31	20.37
60	14.30	18.74	21.29	22.45	19.20
Gas treatment means	21.34	19.42	23.22	22.09	

There were no significant differences
between gas or nitrogen treatments ($P \leq 0.05$)

Table 35. Mean rates of stomatal conductance (μmol.m^{-2} s^{-1}) for adaxial and abaxial leaf surfaces of *Briza media* after being exposed for 11 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
	ADAXIAL LEAF SURFACE				
0	1.45	1.13	1.44	1.47	1.37
20	1.40	1.43	1.41	1.28	1.38
40	1.59	1.57	1.59	1.56	1.58
60	1.41	1.55	1.02	1.31	1.32
Gas treatment means	1.46	1.42	1.37	1.40	

There were no significant differences between gas or nitrogen treatments ($P \leq 0.05$)

	ABAXIAL LEAF SURFACE				
0	1.68	0.88	0.82	0.88	1.67
20	1.15	1.33	0.87	0.98	1.08
40	1.15	1.19	1.04	1.04	1.11
60	1.07	0.79	0.77	0.68	0.83
Gas treatment means	1.26	1.05	0.88	0.90	

L.S.D. ($P \leq 0.05$) between gas treatments = 0.28
There were no significant differences between nitrogen treatments

Footnote: There were no significant differences ($P \leq 0.05$) between treatments for rates of stomatal conductance of adaxial or abaxial leaf surfaces after 13 and 15 weeks exposure (data not shown).

Table 36. Mean rates of stomatal conductance (μmol.m^{-2} s^{-1}) for adaxial and abaxial leaf surfaces of *Agrostis capillaris* after being exposed for 9 weeks to different levels of SO_2 + NO_2 and wet nitrogen applications.

	ppb SO_2 + NO_2				
Nitrogen additions Kg.ha^{-1}.y^{-1}	0	10	20	40	Nitrogen treatment means
	ADAXIAL LEAF SURFACE				
0	0.72	1.19	0.84	0.69	0.86
20	1.01	0.97	1.06	1.38	1.13
40	1.09	1.19	1.20	1.38	1.21
60	0.64	1.32	0.90	1.19	1.01
Gas treatment means	0.89	1.17	1.00	1.16	

L.S.D. ($P \leq 0.05$) between nitrogen treatment means = 0.28
There were no significant differences between gas treatments

	ABAXIAL LEAF SURFACE				
0	0.23	0.43	0.26	0.28	0.30
20	0.34	0.19	0.32	0.34	0.30
40	0.39	0.17	0.28	0.31	0.29
60	2.44	0.40	0.16	0.39	0.30
Gas treatment means	0.30	0.30	0.26	0.33	

There were no significant differences ($P \leq 0.05$)
between gas or nitrogen treatments.

Footnote: There were no significant differences ($P \leq 0.05$) between treatments for rates of stomatal conductance of adaxial or abaxial leaf surfaces after 11 and 13 weeks exposure (data not shown).

Estimates of total deposition rates of sulphur and nitrogen for the different SO_2 + NO_2 treatments for the different exposure periods have been derived from computer models by Professor D Fowler. These are shown with percentage changes in total plant dry weights, compared to control plants (grown in charcoal filtered air), for the different exposure treatments in Table 37. It is apparent that the critical level of SO_2 + NO_2 for adverse effects on the growth of *A. capillaris* is between 10 ppb SO_2 + 10 ppb NO_2 and 20 ppb SO_2 + 20 ppb NO_2 for 11 weeks. This is equivalent to a critical load of between 0.28 – 0.56 $g.m^{-2}$ S + 0.08 – 0.16 $g.m^{-2}$ N as gases.

For *B. media*, there was a transitory stimulation of growth in response to the 10 ppb SO_2 + 10 ppb NO_2 (0.32 $g.m^{-2}$ S + 0.10 $g.m^{-2}$ N deposition after 10 weeks) and 20 ppb SO_2 + 20 ppb NO_2 (0.64 $g.m^{-2}$ S + 0.21 $g.m^{-2}$ N deposition after 10 weeks; 0.78 $g.m^{-2}$ S + 0.28 $g.m^{-2}$ N deposition after 12 weeks) treatments. This was lost after 16 weeks of exposure to the treatments and not found at any stage for plants exposed to the highest 40 ppb SO_2 + 40 ppb NO_2 treatment. Such transitory changes in biomass production (positive or negative) at different stages in plant development may well prove critical in influencing vegetation composition in mixed swards where plants co–exist in different developmental stages. Clearly, there is a need to consider transitional growth changes when assessing critical levels/loads of pollutants.

The responses to SO_2 and NO_2 of *A. capillaris* and *B. media* in this investigation conflict with those found in the dose–response studies in Chapter 6. In the earlier investigation, exposures up to and including 40 ppb SO_2 + 40 ppb NO_2 had no significant effects on plant growth. The previous study started several weeks later in the calendar year and it is possible that sensitivity of plants to pollutants may be influenced by the time of exposure. Temperature, in particular, may influence plant sensitivity to pollutants (Ashenden & Mansfield, 1978). This factor could substantially alter estimates of critical loads and levels of pollutants derived from experimental exposures.

Table 37 Calculated deposition rates of sulphur and nitrogen (g.m^{-1}) from SO_2 + NO_2 gaseous exposures and percentage changes in total plant dry weights, compared to controls (charcoal filtered air = 100%) for *Briza media* and *Agrostis capillaris*.

	ppb SO_2 + NO_2											
	0			10			20			40		
Weeks of exposure	% Dry weight	Total S	Total N	& Dry weight	Total S	Total N	% Dry weight	Total S	Total N	% Dry weight	Total S	Total N
						Briza media						
12	100	–	–	132*	0.32	0.10	155*	0.64	0.21	117	1.29	0.41
14	100	–	–	115	0.39	0.14	126*	0.78	0.28	97	1.56	0.56
16	100	–	–	114	0.45	0.17	117	0.89	0.34	101	1.78	0.69
						Agrostis capillaris						
11	100	–	–	84	0.28	0.08	63*	0.56	0.16	62*	1.11	0.32
13	100	–	–	89	0.34	0.11	58*	0.68	0.23	64*	1.36	0.45
15	100	–	–	107	0.42	0.16	80*	0.84	0.32	61*	1.68	0.63

% changes in dry weight are averaged over all wet deposition treatments. Additional applications of wet nitrogen in the form of ammonium nitrate mist at rates equivalent to 0, 20, 40 and 60 kg.N.ha^{-1}.y^{-1} had no effects on biomass production ($P \leq 0.05$).

* Indicates significant ($P \leq 0.05$) change in % dry weight from control plants.

8. DISCUSSION

The manner in which pollutants affect semi-natural ecosystems is extremely complex. Responses of individual plants to individual pollutants depend on a range of factors, including the concentration and duration of exposure to the pollutant, environmental conditions such as light and temperature, and the physiological status and stage of development of the plant. In field conditions all of these factors fluctuate and interact and it is difficult to make general statements about the impact of pollutants on complex communities of plants which will apply in all situations. The concepts of critical levels and critical loads, while convenient for setting emission standards, need substantial refinement for a realistic estimate of the threat of pollutants to terrestrial ecosystems. These refinements need to consider the differential responses of species to the complex matrix of stresses to which plants are exposed in their native environments and the plant – plant interactions within the community (see Caporn, in press).

The first phase of the research clearly demonstrated that wet acid deposition in the form of mists, may alter plant growth rates. Three of the four species exposed to the misting treatments showed increased dry matter production at the most acid pH 2.5 treatment suggesting that, some species could utilise sulphur and nitrogen in wet deposition as plant nutrients. In contrast, *Lotus corniculatus*, the only legume species tested, showed decreased growth in the more acid pH 3.5 and pH 2.5 treatments compared to ones exposed to pH 4.5 or pH 5.6 mists. Similarly, some species in the combined gas x mist studies showed decreased dry weight accumulation in response to increasing acidity of mists over the range of gas exposure treatments including charcoal–filtered air (unpolluted gas, control treatment). These observations are similar to those reported for crop species where most are unaffected except at high levels of acidity (see reviews by Irving, 1983; Evans, 1984). In this connection, it is interesting to note that leguminous crop species have been found to be sensitive to lower levels of acidity (Evans et al., 1982 & 1983; Ashenden & Bell, 1989).

The influence of soils on plant sensitivity to acid mists has not been investigated in this programme. However, previous work has shown that the sensitivity of plants to acid rain may vary according to the soils on which they grow and that differences in plant sensitivity are not related to the expected sensitivities of the soils (Ashenden & Bell, 1987a and b).

Many of the species studied were found to be resistant to the gas pollution treatments. Indeed, plants of *Nardus stricta* grew largest in the triple pollutant combination of SO_2 + NO_2 + O_3. However, two of the species used in the larger gas x mist exposure studies (*Lolium perenne* and *Trifolium repens*) suffered substantial dry weight yield reductions in all air pollution treatment compared to plants grown in charcoal–filtered air. For these sensitive species, the greatest dry weight depressions were found for plants exposed to the triple pollutant combination of SO_2 + NO_2 + O_3 rather than for the SO_2 + NO_2 or O_3 alone treatments. Clearly, there is an implication that the presence of O_3 may affect the estimation of critical levels/loads of S + N for some species.

Some interactive effects of the mists x gaseous pollution treatments were found on the yields of some species. Generally these showed any increased or decreased dry weight production in response to high acid mist treatments (eg. pH 2.5) were overridden by the more harmful air pollution treatments, particulary the combination of SO_2 + NO_2 + O_3. The inconsistent responses of plants to combinations of pollutants are comparable with previous reports by other researchers. Exposures to combinations of pollutants may cause less than additive, additive or synergistic effects on the growth of different species compared to responses to pollutants applied singly (Ashenden & Mansfield, 1978; Mooi, 1984; Ernst et al., 1985, Wolting & van Remortal, 1985; Whitmore, 1985; Wright et al., 1986). There is no general pattern of plant response.

In terms of critical loads, it is apparent that these are substantially different for gaseous as compared to wet inputs to vegetation. Generally, species are more sensitive to gases. This is no doubt because gases may pass more readily through the stomata of leaves and come into contact with internal leaf structures. An extreme example was the case of *Lolium perenne* where $70.1 Kg.ha^{-1} S + 61.2 Kg.ha^{-1} N$, as wet deposition, increased biomass two-fold while $20.0 Kg.ha^{-1} S + 8.1 Kg.ha^{-1} N$ as gases reduced biomass.

Studies aimed at defining dose-response relationships for plants exposed to different concentrations of $SO_2 + NO_2$ again showed substantial differences between species. For six of the ten species, there were no significant effects of the pollution treatments up to and including 40ppb SO_2 + 40ppb NO_2. The remaining species showed some growth stimulation at the intermediate pollution treatments (10 and 20ppb $SO_2 + NO_2$) with a reversal of response at the highest 40ppb SO_2 + 40ppb NO_2 treatment, when the biomass of plants was not significantly different from controls. The fitting of dose-response curves for these species suggested a maximum growth stimulation at 13-15 ppb $SO_2 + NO_2$. This stimulation is likely to be a fertilizer effect whereby the plants are able to utilise the nitrogen (and possibly the sulphur) from the pollutant gases. At higher levels of exposure, the fluxes of pollutants into the plants exceed levels at which they may be detoxified.

The stimulation of growth by low levels of pollutants has been found by other researchers (see review by Ashmore, Bell & Rutter, 1988). However, there are substantial discrepancies between individual studies. In this connection, it is important to point out that two of the species which were found to be unaffected by the pollution treatments in this phase of the programme were found to have contrasting growth responses to $SO_2 + NO_2$ in a later experiment (Phase 3). *Agrostis capillaris* showed significant reductions in biomass at concentrations of 20ppb SO_2 + 20ppb NO_2 and above while *Briza media* showed growth stimulation at both 10ppb SO_2 + 10ppb NO_2 and 20ppb SO_2 + 20ppb NO_2 compared to controls and plants exposed to 40ppb SO_2 + 40ppb NO_2. It is suggested that the stage of plant development and time of year (particularly in relation at ambient temperatures) may exert a substantial influence on the sensitivity of individual species to aerial pollutants, Previous work has shown that the sensitivity of plants to pollutants may vary according to the stage of development (Caporn & Hutchinson, 1986) and time of year when exposed (Whitmore, 1985). These are important considerations which need to be addressed in the refinement of estimates for critical levels and loads of pollutants for vegetation.

Wet nitrogen applications, in the form of ammonium nitrate, were not found to affect plant growth in the studies reported here. However, experiments were only conducted over short time periods (up to 16 weeks). It is possible that longer-term exposures may result in growth alteration. This may be particularly important with respect to upland species which grow on poorly buffered soils.

Both positive and negative growth responses of plants to the different pollution treatments have been recorded during this experimental programme. The smallest inputs of S + N found to significantly ($P \leq 0.05$) stimulate growth were 3.2 $kg.ha^{-1}$ S + 1.0 $kg.ha^{-1}$ N as gaseous SO_2 + NO_2 (10 ppb of each gas for 10 weeks) and 1.4 $kg.ha^{-1}$ S + 1.3 $kg.ha^{-1}$ N as wet deposition (applied as pH 4.5 mist for 18 weeks). The smallest inputs of S + N found to significantly ($P \leq 0.05$) reduce growth were 5.6 $kg.ha^{-1}$ S + 1.6 $kg.ha^{-1}$ N as gaseous SO_2 + NO_2 (20 ppb of each gas for 11 weeks) and 7.7 $kg.ha^{-1}$ S + 6.6 $kg.ha^{-1}$ N as wet deposition (applied as pH 3.5 mist for 18 weeks). By definition, the critical load of a pollutant (or combination of pollutants) is the quantity above which there is a harmful effect on a sensitive component of the ecosystem. However, positive growth responses as well as growth inhibition caused by pollutants may be expected to cause changes in vegetation structure if not all species in a community respond in the same manner. Hence, even the low levels of pollutants found to alter plant growth in these studies may be critical in terms of causing changes in vegetation patterns.

It is clear that future research needs to characterise the responses to combinations of pollutants for a broad range of species at different stages of development if the most sensitive components of ecosystems are to be identified. The additional influences of temperature, soils and changing climate on species-specific responses need evaluation : these factors will substantially alter the levels and loads of pollutants which affect plant growth. Finally, there is an urgent need to move to studies on mixed species swards in order to test the validity of extrapolating from studies on single species to complex plant communities.

9. CONCLUSIONS

The broad range of studies conducted during this research programme give rise to a number of important conclusions in relation to defining critical loads and levels of combined sulphur and nitrogen for semi-natural ecosystems in the UK.

1. Species vary considerably in their responses to S and N applied both as acidified mists and gaseous SO_2 and NO_2. While low inputs of S and N may result in growth stimulation, no generalised across-species relationship has been established. Where there is a growth stimulation with low and intermediate levels of pollutants, this is reversed with higher levels of deposition.

2. Limited evidence suggests that leguminous plants are more sensitive to acid mists than those of other plant families. Most species tested showed no effects or growth stimulation with high inputs of S + N even in highly acidic pH 2.5 mist. In contrast, for *Lotus corniculatus*, applications of pH 4.5 mist (1.4 $kg.ha^{-1}$ S + 1.3 $kg.ha^{-1}$ N deposited) stimulated growth while pH 3.5 (7.7 $kg.ha^{-1}$ S + 6.6 $kg.ha^{-1}$ N deposited) reduced growth relative to control plants (pH 5.6 mist; 0.9 $kg.ha^{-1}$ S + 0.8 $kg.ha^{-1}$ N deposited). This observation relates well to previous studies on leguminous crop plants, some of which were adversely affected by exposure to pH 4.5 mists.

3. The presence of ozone may affect critical levels/loads of S + N for some species. Exposures to O_3 + SO_2 + NO_2 may negate or promote stimulation of growth compared to exposure with SO_2 + NO_2. For other species, the triple pollutant combination may result in additive or less than additive growth reductions compared to separate exposures to O_3 and SO_2 + NO_2.

4. Critical loads of S + N are substantially different for gaseous as compared to wet inputs to vegetation. Generally, species are more sensitive to gases. An extreme example was for *Lolium perenne* where 70.1 $kg.ha^{-1}$ S + 61.2 $kg.ha^{-1}$ N, as wet deposition, increased biomass two-fold. Conversely plant biomass was reduced by exposure to SO_2 + NO_2 at 20.0 $kg.ha^{-1}$ S + 8.1 $kg.ha^{-1}$ N and SO_2 + NO_2 + mist applications up to 89.3 $kg.ha^{-1}$ S + 68.5 $kg.ha^{-1}$ N.

5. Wet N applications, in the form of ammonium nitrate, up to 18.5 $kg.ha^{-1}$ N deposited (equivalent to 60 $kg.ha^{-1}.y^{-1}$ N) have little effect on plant growth in short-term exposures (up to 16 weeks). However, it is possible that longer-term exposures, especially for plants growing on poor native soils may result in growth alteration (not researched).

6. It is considered that the stage of plant development, time of year and temperature may exert a substantial influence on critical loads and levels of pollutants for individual species. Contrasting growth responses to SO_2 + NO_2 exposures were found for different experiments on some species. Some growth responses were transitional in their occurrence.

7. The influence of soil-type on plant responses has not been considered. However, earlier research on the impact of acid rain on plants revealed substantial differences

in responses of plants grown on different soils which were not related to the expected sensitivities of the soils to acid deposition. Further research is suggested in this area.

8. It is considered that both positive and negative growth responses of plants to pollutants may be critical in terms of altering vegetation patterns in mixed species swards. The smallest inputs of S + N found to significantly ($P \leq 0.05$) stimulate growth in this programme were 3.2 $kg.ha^{-1}$ S + 1.0 $kg.ha^{-1}$ N as gaseous SO_2 + NO_2 (10 ppb of each gas for 10 weeks) and 1.4 $kg.ha^{-1}$ S + 1.3 $kg.ha^{-1}$ N as wet deposition (applied as pH 4.5 mist for 18 weeks). The smallest inputs of S + N found to significantly ($P \leq 0.05$) reduce growth were 5.6 $kg.ha^{-1}$ S + 1.6 $kg.ha^{-1}$ N as gaseous SO_2 + NO_2 (20 ppb of each gas for 11 weeks) and 7.7 $kg.ha^{-1}$ S + 6.6 $kg.ha^{-1}$ N as wet deposition (applied as pH 3.5 mist for 18 weeks).

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