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Effects of elevated CO_2, O_3 and K deficiency on Norway spruce (*Picea abies*): nutrient supply, content and leaching

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SUMMARY

Two clones of 5-yr-old Norway spruce (*Picea abies* [L.] Karst.) were exposed to two atmospheric concentrations of CO_2 (350 and 750 μmol mol^{-1}) and of O_3 (20 and 75 nmol mol^{-1}) in a phytotron at the GSF-Forschungszentrum (Munich) over the course of a single season (April–October). The phytotron was programmed to recreate an artificial climate similar to that at a high elevation site in the Inner Bavarian forest, and trees were grown in 40 l containers of soil (pH 3·5) fertilized to achieve two levels of potassium nutrition; well fertilized and K-deficient. Foliar nutrient analyses performed at the beginning of the exposure indicated that the fertilization programmes achieved their goal without significantly altering the levels of other nutrients or the soil pH. At the beginning of the fumigation, foliar K concentrations were 7–9 mg g^{-1} d. wt for well fertilized trees and 4–5 mg g^{-1} d. wt for trees receiving no supplemental K. Over the course of the season, differences between K treatments intensified so that by the end of the experiment there was a five to sixfold difference between foliar K concentrations. This was associated with slight, but significant ($P < 0{\cdot}05$), decreases in S and Zn (and of Cu in the 1989 needle year age class) and higher levels of C, N and Mg in K-deficient trees. Foliar N concentrations were low for all trees (9–15 mg g^{-1} needle d. wt) but were similar to levels found in the field.

Elevated O_3 was found to decrease significantly the C ($P < 0{\cdot}05$) and N ($P < 0{\cdot}001$) content of both current-year (1989) and previous-year (1988) needles independent of CO_2 concentration, but apart from some minor changes in the concentrations of Cu and Mn in the current-year needles no other effects of the pollutant on plant nutrient status were found. In contrast, CO_2 enrichment resulted in significantly ($P < 0{\cdot}01$) lower concentrations of K and P (effects on Mg were also on the borderlines of statistical significance) in current-year needles, but there was no influence on the nutrient composition of the previous-year needles (although effects on N were on the borderlines of statistical significance). CO_2 enrichment also increased ($P < 0{\cdot}05$) the C:N ratio of both current-year and previous-year needles. One factor contributing to the decline in foliar K at elevated CO_2 appeared to be a marked increase (25–30%) in the rate at which cations were leached from the canopy by repeated simulated acid mist (pH 4·0) events, and this effect occurred independently of the O_3 concentration. The information presented will aid the interpretation of parallel studies examining the effects of elevated CO_2 and/or O_3 on seasonal changes in photosynthesis, non-structural carbohydrate content, antioxidants, tree growth and water use efficiency, and sheds further light on the growing scepticism concerning the role of O_3 in the development of Mg and K-deficiency symptoms characteristic of certain types of forest decline in central Europe.

Key words: Elevated CO_2, O_3, nutrient status, nutrient leaching, *Picea abies* (Norway spruce).

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INTRODUCTION

Anthropogenic activities are causing the tropospheric concentrations of carbon dioxide (CO_2), ozone (O_3) and a number of other trace gases to rise at a steadily increasing rate (Penkett, 1988; Keeling *et al.*, 1989). This has prompted considerable interest in the potential impact of atmospheric change on natural and managed ecosystems. Much work has concentrated on answering the question of how crops will respond to rising CO_2 (e.g. Lemon, 1983; Kimball *et al.*, 1990) and to higher O_3 (Davison & Barnes, 1992; Jäger *et al.*, 1992), but less attention has been paid to the long-term effects of elevated CO_2 on trees (see Eamus & Jarvis, 1989; Ceulemans & Mousseau, 1994) and very little is known about the way in which responses to rising CO_2 might be affected by simultaneous increases in O_3, and vice versa (Allen, 1990; Barnes, 1993). This represents a serious gap in our current knowledge, since trees and temperate forest ecosystems account for a large proportion of terrestrial atmospheric carbon exchange and influence the hydrology of large areas (Waring & Schlesinger, 1985). It is essential that we understand how forest productivity will be affected by increasing concentrations of CO_2 and O_3, if we are to be able not only to provide better predictions of future global carbon budgets but also to decide upon policies for the future management of forested ecosystems.

Short-term exposure of a wide variety of tree species to elevated CO_2 has been shown to increase net photosynthesis and plant productivity (Eamus & Jarvis, 1989; Ceulemans & Mousseau, 1994). However, there is growing recognition that this response can only be sustained where other resources, particularly nutrients, are available to support the extra growth induced by CO_2 enrichment (Kirschbaum *et al.*, 1994; Gundersson & Wullschleger, 1994; Petterson & McDonald, 1994; Barnes *et al.*, 1995*a*). One factor influencing the nutrient content of tree foliage is the rate at which cations are leached from the canopy (Tukey, 1970). Several factors are known to influence this process, including the pH, the frequency, duration and intensity of precipitation, as well as the proportion of the foliage surface in contact with a given volume of water (Riederer, 1989; Barnes & Brown, 1990). In conifers, leaching is reduced to a minimum by the hydrophobic nature of the needle surface and the presence of a relatively thick and impermeable cuticle (Martin & Juniper, 1970). However, many factors, including CO_2 (Prügel, 1994), O_3 (Wellburn *et al.*, 1996), acid rain/mist (Riederer, 1989) and nutrient status (Chiu *et al.*, 1992; Ylimarto *et al.*, 1994) are known to influence the nature and functioning of the cuticle and associated epicuticular waxes, as well as to affect membrane permeability (Heath & Castillo, 1988; Marschner, 1986). This suggests that increased foliar leaching under the combined action of elevated CO_2+O_3 and possibly nutrient deficiency, could exacerbate the direct effects of CO_2 (Oberbauer *et al.*, 1986) and O_3 (Ogner, 1993) on foliar nutrient status, and therefore influence long-term CO_2 responsiveness. This paper reports the results of a season-long study on Norway spruce (*Picea abies* [L.] Karst.) designed to examine this hypothesis. Cloned trees were exposed in state-of-the-art controlled environment facilities to elevated CO_2 and/or O_3 over the course of a single growing season and effects on soil nutrient availability, nutrient status and leaching were examined. Particular attention was paid to the impact of O_3 on foliar leaching, since the combined action of O_3 and acid mist has received much attention in recent years in relation to the development of forest decline in mountainous areas of central Europe and eastern North America (Bosch *et al.*, 1986; Roberts, Skeffington & Blank, 1989; Taylor, Johnson & Andersen, 1994).

MATERIALS AND METHODS

Tree culture

Two clones (numbers 399 and 773) of Norway spruce (*Picea abies* [L.] Karst.) originating from a mid-altitude provenance of trees growing in the Bavarian forest (Schongau, provenance number 84019) were provided by the Bavarian Forest Service. In April 1987, when the trees were 3-yr-old, they were lifted from the nursery bed, their roots washed and pruned, and planted four trees per pot in large pots (40 dm^3) containing an acidic (pH 3·5) sandy forest soil derived from granite (A_h and B_v-horizons of a dystric Cambisol), which had previously supported the feeder roots of an old forest stand near Speyer (Rhineland–Pfalz). Trees were grown outdoors at Freising Dürnast (Experimental Station of the Institute of Plant Nutrition, Technical University of Munich), situated in a 'clean air' region of Bavaria ($\approx$ 30 km north of Munich), and selectively fertilized with KNO_3 and K_2SO_4 between August 1987 and March 1989 with the aim of achieving two levels of K nutrition; well fertilized and K-deficient (see Pfirrmann, 1992). One tree per pot was harvested for analytical purposes. The remaining trees were exposed to controlled levels of CO_2 and/or O_3.

Chamber conditions and exposure details

On 6 April 1989 trees were randomized according to size and transferred to the GSF phytotron (see Payer *et al.*, 1986*a*, *b*) where they were installed in temperature-controlled compartments providing root temperatures comparable to those under field conditions (see Blank *et al.*, 1990). Into each of four walk-in environment chambers were placed 16 pots

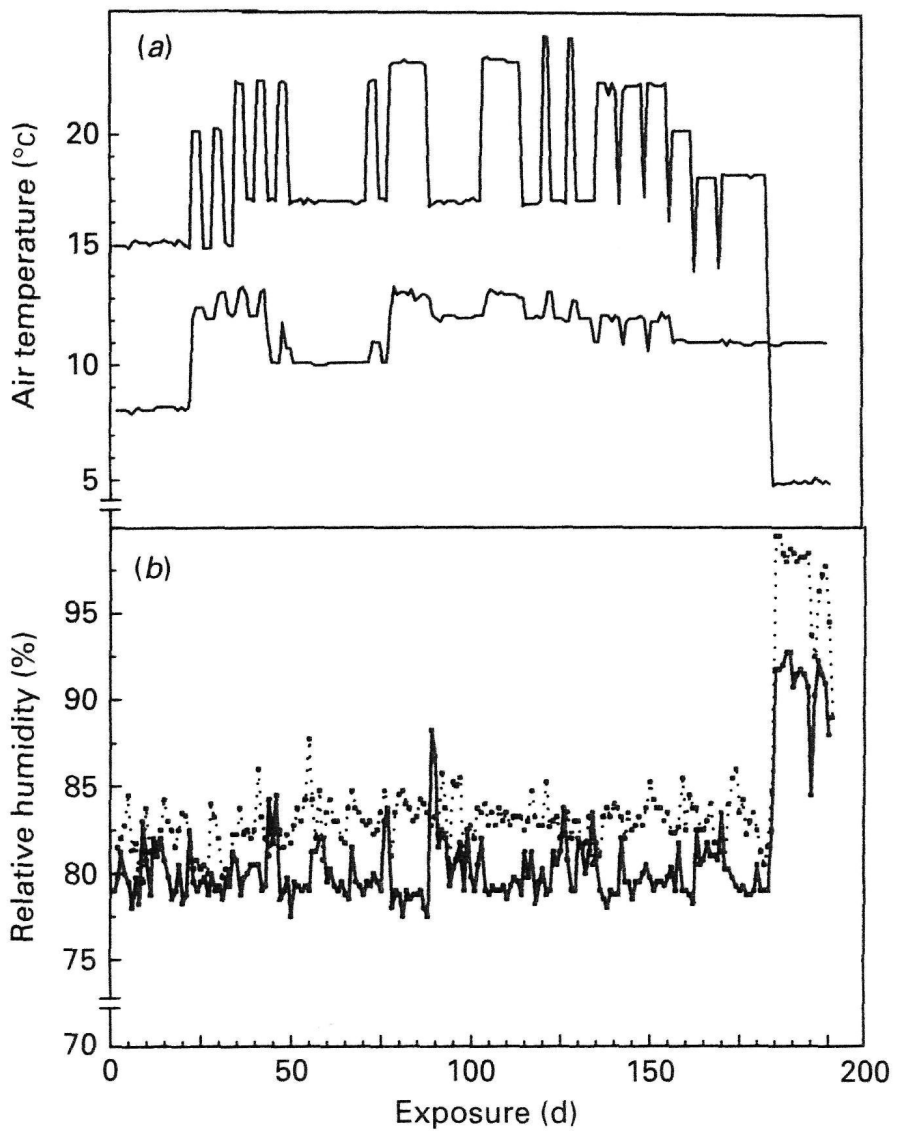

Figure 1. (*a*) Daily maximum and minimum air temperatures and (*b*) relative humidity of the four chambers comprising the phytotron. Air temperature was based on 20 yr of continuous monitoring at meteorological stations based at high-elevation (≈ 800 m above sea level) in the Inner Bavarian Forest. Relative humidity was adjusted to a daytime minimum 80 ± 8 % throughout the exposure period in order to provide undisturbed conditions for stomatal gas exchange. Only during mist periods were higher humidities achieved (final stages of exposure). Individual chamber conditions were virtually identical.

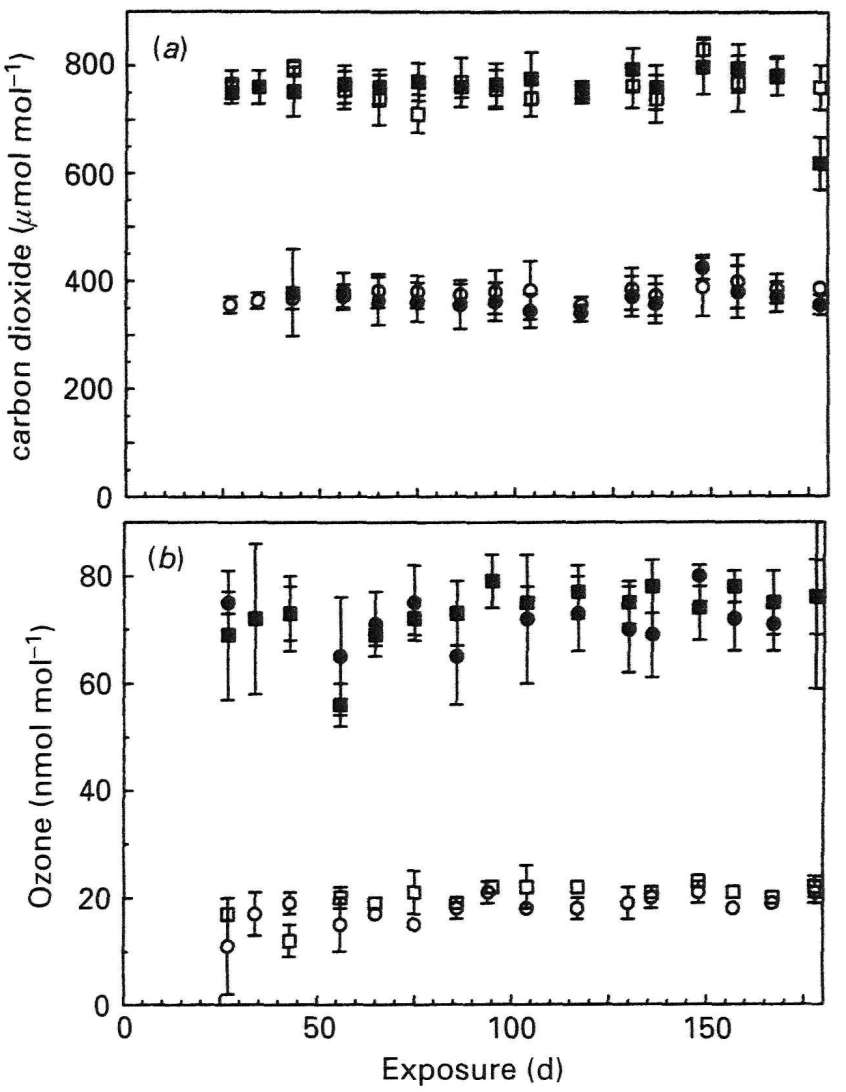

Figure 2. Weekly mean May–October atmospheric CO_2 and O_3 concentrations (± average daily variations) inside the four experimental chambers which comprise the phytotron. CO_2 treatments ambient (circles) and elevated (squares), O_3 treatments 'non-polluted air' (open symbols) and 'polluted air' (closed symbols). Chambers were initially ventilated with clean ambient air before starting the treatments on day 26.

(each containing three trees). At this stage, all chambers were maintained at 15 °C day/8 °C night and ventilated with clean (charcoal/Purafil®-filtered) ambient air. After a 26 d period of 'equilibration', CO_2 and O_3 treatments were superimposed on an entirely artificial climate, recreating conditions characteristic of a high elevation site in the Inner Bavarian Forest. The software used to recreate climatic patterns was developed in collaboration with the Institute of Bioclimatology at the University of Munich and was based on data from 20 yr of continuous monitoring at stations belonging to the German Meteorological Office (DWD; see Payer *et al.*, 1986*b*; Blank *et al.*, 1990). Essentially, the programme consisted of diel changes in light and temperature conditions alternating every 2–7 d on a rotating pattern between cloudy, hazy and sunny days with maximum photon flux densities (PFDs) at midday of 500, 900 and 1150 μmol m^{-2} s^{-1}, respectively (Barnes & Pfirrmann, 1992). Air temperature in the chambers was monitored continuously and was controlled on a dynamic basis between a midday maximum of 23 °C in August and a night-time minimum of 8 °C (±0·5 K) in April and October (Fig. 1*a*). The relative humidity was maintained at 80 ± 8 % in order to provide undisturbed conditions for stomatal gas exchange. Only during misting periods were higher humidities achieved (see Fig. 1*b*). Radiation was provided by metal-halide, xenon-arc and krypton floodlamps producing a spectrum close to that occurring in the field (between 350 and 800 nm). Radiation in the near-ultra-violet (u.v.-A: 320–380 nm) was ≈ 50 % lower than that under field conditions, u.v.-B (280–320 nm) was less than 10 % of that found in the field, and no u.v.-C was detectable (< 280 nm). PFDs lower than found in the field were compensated for by extending the period of maximum (noon time) illumination. In this way, cumulative PFDs comparable to those in field conditions were achieved. Between May and October, air temperature, relative humidity and PFD were virtually identical in the four chambers. Trees were watered by a computer-controlled irrigation system governed by data acquired from tensiometers placed in each of the pots. This system was programmed to re-water the plants to field capacity when the soil water tension dropped to 150 hPa (Pfirrmann, 1992). Evaporation from the soil surface was prevented by fitting a polyethylene cover over

the surface of each pot. These covers also prevented the percolation of throughfall into the soil.

Between 2 May and 24 October two chambers were maintained at ambient CO_2 by ventilating with filtered air drawn from outside the building, whilst the other two chambers were maintained at elevated CO_2 by injecting CO_2 into the ambient air stream from cylinders to maintain a target atmospheric CO_2 concentration of 300 μmol mol^{-1} above external concentrations (i.e. target concentration *c.* 750 μmol CO_2 mol^{-1} dry air). The atmospheric CO_2 concentration in the 'ambient' chambers was slightly higher (on average $\approx$ 5 %) than background levels owing to the presence of researchers and technicians caring for plants and undertaking experimental duties within the chambers. Ozone, produced by electric discharge from pure oxygen, was continuously injected into individual chambers to maintain target O_3 concentrations of 20 nmol mol^{-1} ('non-polluted' air) or 75 nmol mol^{-1} ('polluted' air) for 24 h d^{-1} (see Fig. 2). SO_2 and NO_2 were injected into all the chambers to provide a constant background level of 8–15 nmol mol^{-1} for 24 h d^{-1}. Comprehensive details of gas-control systems and analytical methods are provided elsewhere (Payer *et al.*, 1986*a*, *b*).

Soil analysis

Before the beginning (March, 1989) and at the end of the experiment (October, 1989) pots in which the two clones had been growing were selected at random for soil analyses. Soil was removed from a depth of 0–5 cm, separated as described in Pfirrmann *et al.* (1990) and the d. wt recorded after 2 d at 105 °C. Extracts of fine soil were shaken overnight in 0·1 M KCl, allowed to settle for 2 h and then the soil-pH was determined using a glass electrode. Exchangeable cations were determined in percolates of 2·5 g fine soil and 100 ml of unbuffered 1 M NH_4Cl as described by Meiwes *et al.* (1984). The concentrations of Al, K, Ca, Fe, Mg, Mn and Na in percolate samples of fine soil were determined by ICP-analysis (see Schramel, 1988). All analyses were carried out on two replicates of each sample. Effective cation exchange capacity (CECeff) was calculated from the sum of the equivalents of the exchangeable cations (Meiwes *et al.*, 1984).

Tissue nutrient analysis

Current- [1989] and previous [1988]-year needles were harvested at the beginning (April, 1989) and end of the experiment (October, 1989) from the uppermost whorl of one tree in each pot. One tree per pot was removed prior to budburst and trees of clone 773 were destructively sampled following exposure to a sequence of acid mists in October. Fine root sample (< 2 mm diam.) were extracted from just beneath the soil surface at six positions marked at random on the surface of each pot. Plant material was washed in deionized water, cleaned, dried to constant weight in an oven (65 °C) and then ground. P, S, K, Ca, Mg, Al, Cu, Fe, Mn and Zn contents were determined by ICP analysis (Schramel, 1988), and those of C and N using a Carlo Erba analyser (Steffen & Schramel, 1988).

Throughfall chemistry

Towards the end of the exposure period (days 160–172) all trees were exposed to artificial acid mist (eight events). Mist events consisted of applying deionized water enriched with $(NH_4)_2SO_4$ (18 mg l^{-1}), adjusted to pH 4·0 with H_2SO_4, for 6 min in every 10, for 12 h overnight, with the mist applied from glass spray units in the roof of each chamber (see Blank *et al.*, 1990; Pfirrmann *et al.*, 1990). Throughfall was collected in perspex trays connected to the lid of each pot, and 25 ml aliquots were analysed for K, Mg and Ca as described in Pfirrmann *et al.* (1990), correcting for the levels of specific elements in 'blank' leachates collected from nylon 'trees'.

Statistical analyses

The experiment involved the exposure of 192 trees to controlled levels of CO_2 and O_3, the smallest possible cell comprising two pots holding six plants. The influence and interaction of CO_2, O_3, K status and clone were determined by analysis of variance (ANOVA) using linear models for unbalanced data because of missing values. After each ANOVA, the residuals were tested for normal distribution. Since it was not possible to assign root samples to individual trees within each pot, these data were analysed using the 'pot' as the unit of replication. Time-course data were first subjected to a repeated measures ANOVA (RM-ANOVA) model to test the effects of time, group factors and interactions between them (see Barnes *et al.*, 1995*a*). All effects involving the factor 'time' were tested by the univariate *F*-test method, with adjustment for the degrees of freedom (Geisser & Greenhouse, 1958). This indicated that only two- and three-way interactive effects of time with CO_2 and O_3 were significant ($P < 0{\cdot}05$), so the data were re-analysed using a reduced multivariate ANOVA (MANOVA) model concentrating only on those factors which were significant. Finally, the data gathered at each sampling time were subjected to an ANOVA. Because of the complex experimental design and the length of the fumigation it was not possible to repeat

treatments in different chambers, so all analyses were made on the assumption that two plants grown in different chambers receiving the same treatment were likely to be as different as (or as similar to) plants in the same chamber (see Barnes & Pfirrmann, 1992). This assumption was supported by the results of a preceding experiment in which spruce trees were exposed to a combination of O_3 and acid mist, using an experimental design in which trees were treated in replicated chambers (Blank *et al.*, 1990).

RESULTS

Soil analyses

In March 1989, analyses revealed the principal difference achieved by the contrasting fertilization procedures to be the level of soil potassium. Well fertilized soil exhibited a level of exchangeable K of 3·65 μmol g^{-1} fine soil, 65 % higher than that of soil receiving no supplemental K (2·21 μmol g^{-1} fine soil). Analyses showed that neither soil pH nor the availability of any other element was significantly affected by the fertilization treatment.

Plant growth, and additional fertilization during the period of the experiment intensified the differences between K treatments, so that by October 1989 the level of exchangeable K in soil receiving supplemental fertilization was ≈ 3·5-fold higher than that of soil which received no K fertilizer. There was also *c.* 30 % difference in the levels of soil sulphur induced by the contrasting fertilization regimes (data not shown). Table 1 shows the results of soil nutrient analyses conducted in October 1989. Three and four-way interactions have been omitted as no significant effects were found at this level of analysis. The data reveal that contrasting K fertilization programmes achieved their goal. There were no significant differences between fertilization treatments in exchangeable Al, Ca and Mg (nor in Mn and Na – data not shown), but fertilization during the period of the experiment resulted in 20–25 %

Table 1. *Summary of soil analyses on NH_4Cl percolates of fine soil following a season-long exposure of Norway spruce*[1] (Picea abies) *to elevated CO_2 (750 vs. 350 μmol mol^{-1}) and/or O_3 (75 vs. 20 nmol mol^{-1}) superimposed on an entirely artificial climate recreating that at a high altitude site in the Bavarian Forest*

Treatment			Soil nutrient composition						
O_3	CO_2	Soil	Al	K	Mg	Ca	Fe	CECeff*	pH
20	350	+K	34·3	3·71	7·3	5·8	2·77	57·2	3·53
20	350	−K	27·2	0·15	6·7	4·2	2·29	43·9	3·53
20	750	+K	37·5	3·98	9·6	11·5	3·23	69·4	3·52
20	750	−K	40·2	1·11	7·2	7·9	2·61	61·9	3·56
75	350	+K	34·0	5·61	4·0	4·5	5·08	56·6	3·52
75	350	−K	34·8	1·88	12·5	8·3	2·36	63·0	3·53
75	750	+K	34·9	3·65	12·4	7·4	3·57	65·8	3·50
75	750	−K	37·9	1·39	6·4	4·9	2·94	53·4	3·54
			Effects						
O_3			n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.
CO_2			**	n.s.	n.s.	(*)	n.s.	*	n.s.
$O_3 \times CO_2$			*	n.s.	n.s.	*	n.s.	*	n.s.
Soil			n.s.	**	n.s.	n.s.	***	(*)	n.s.
$O_3 \times$ soil			n.s.	n.s.	(*)	n.s.	*	n.s.	n.s.
$CO_2 \times$ soil			*	n.s.	n.s.	n.s.	(*)	n.s.	n.s.
Clone			***	n.s.	n.s.	n.s.	***	**	**

Soil was collected from the A_h and B_v horizons of a hydristic cambisol which had previously supported the feeder roots of an old forest stand in the Rhineland–Pfalz region. Over a 3-yr period, fertilizer amendments were made with the aim of achieving two levels of K nutrition evaluated through soil effects; well fertilized (+K), potassium-deficient (−K). Nutrient concentrations expressed as μmol g^{-1} fine soil d. wt.

Significance of main effects: n.s., not significant, $*P < 0{\cdot}1$; $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

[1] Data represent the mean for two clones – interactions between clone and other factors were not significant.

* Total effective cation exchange capacity = equivalent sum of Al, Ca, Fe, K, Mg, Mn and Na.

Table 2. *Effects of a season-long exposure to elevated CO_2 (750 vs. 350 μmol mol^{-1}) and/or O_3 (20 vs. 75 nmol mol^{-1} 24 h d^{-1}) on the nutrient composition of fine roots (< 2 mm diam.) of Norway spruce* (Picea abies *clone 773) grown in a native forest soil fertilized to achieve two levels of potassium nutrition; well fertilized (+K), potassium-deficient (−K)*

Treatment			Fine root nutrient composition							
O_3	CO_2	Soil	N	K	Mg	Ca	S	P	Zn	Mn
20	350	+K	9·5	17·5	1·20	0·54	358	3·98	56·9	22·5
20	350	−K	10·5	4·8	0·95	1·16	153	2·83	49·1	53·0
20	750	+K	10·2	15·8	0·95	0·42	175	3·87	44·6	20·7
20	750	−K	7·4	3·2	0·95	1·34	107	1·82	71·0	51·5
75	350	+K	11·3	20·1	0·98	0·63	380	4·75	46·2	25·4
75	350	−K	11·1	4·0	0·94	1·17	304	2·35	53·0	40·1
75	750	+K	9·8	12·9	1·15	0·51	267	3·51	56·7	22·9
75	750	−K	10·6	4·8	0·87	0·74	209	2·45	94·3	30·6
			Effects							
O_3			n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
CO_2			n.s.	**	n.s.	n.s.	**	n.s.	n.s.	n.s.
$O_3 \times CO_2$			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil			n.s.	***	n.s.	**	**	**	n.s.	**
Soil × O_3			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	(*)
Soil × CO_2			n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$O_3 \times CO_2 \times$ soil			n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Element concentrations: N, K, Mg, Ca, P (mg g^{-1} d. wt); S, Zn, Mn (μg g^{-1} d. wt).

Significance of main effects: n.s., not significant; *$P < 0{\cdot}1$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

higher ($P < 0{\cdot}001$) levels of Fe by the time of the final harvest, although all pots received equivalent amounts of Fe during fertilization. The contrasting fertilization treatments were reflected in slight (15–25 %) differences in total effective cation exchange capacity (CECeff; $P < 0{\cdot}01$). There were also significant ($P < 0{\cdot}01$) effects of clone on soil pH and on the availability of certain nutrients (e.g. higher levels of exchangeable Al and Fe in the soil supporting clone 399) and they were reflected in significant differences in CECeff between clones (see Table 1). Growth at elevated CO_2 and/or O_3 affected the availability of certain nutrients in soil; O_3 exposure significantly ($P < 0{\cdot}01$) increased the level of exchangeable Fe, whilst elevated CO_2 increased Al ($P < 0{\cdot}01$) and CECeff ($P < 0{\cdot}05$).

Fine-root nutrient content

The influence of season-long exposure to elevated CO_2 and/or O_3 on the nutrient composition of fine roots of clone 773 is shown in Table 2. Major differences in fine-root nutrient composition were associated with soil fertilization regime; K fertilization resulting in significantly higher concentrations of K, S and P and lower concentrations of Al, Ca and Mn. However, significant effects of O_3 and CO_2 on specific nutrients were also found; O_3 exposure increasing the S concentration in fine roots, whilst elevated CO_2 decreased Al, K and S concentrations.

Foliar nutrient analyses

Needle nutrient composition was determined before and after season-long exposure to elevated CO_2 and/or O_3. There were significant differences between clones, but both responded to CO_2, O_3 and K fertilization in approximately the same way so data are presented and discussed independently of clone (see Table 3). Needle K content reflected the contrasting programmes of fertilization. Prior to budburst in 1989, the K content of the previous-year needles was 7–9 mg K g^{-1} needle d. wt for well fertilized trees (+K), and 4–5 mg K g^{-1} needle d. wt for trees grown in K deficient soil (−K). Over the course of the experiment these differences intensified (see Tables 3, 4). Foliar nutrient analyses performed in April 1989 revealed similar levels of all elements, other than K, in trees grown in the two soils. However, by the end of the experiment (October 1989), K deficiency was associated with significantly (30 %) lower concentrations of S and Zn (and of Cu in 1989 needles) and significantly ($P < 0{\cdot}01$) higher levels of C, N and Mg (Tables 3, 4).

O_3 exposure resulted in a significant decrease in the C ($P < 0{\cdot}05$) and N ($P < 0{\cdot}001$) contents of current-year and previous-year needles independent of CO_2 concentration, and in the current-year needles the effect was associated with an increase in Cu and a decrease in Mn content (Tables 3, 4). O_3 exposure had no significant influence on needle

Table 3. *Effects of a season-long exposure to elevated CO_2 (750 vs. 350 µmol mol^{-1}) and/or O_3 (75 vs. 20 nmol mol^{-1} 24 h d^{-1}), superimposed on an entirely artificial climate re-creating that at a high elevation site in the Bavarian Forest, on the nutrient composition of current [1989] year needles of Norway spruce* (Picea abies)

Treatment			Nutrient composition of 1989 needle year										
O_3	CO_2	Soil	C	N	K	Mg	Ca	S	P	Cu	Zn	Fe	Mn
20	350	+K	474	15·4	15·6	1·94	5·11	503	2·58	1·54	35·3	47·5	385
20	350	−K	489	13·4	1·9	2·02	3·29	327	1·87	1·05	18·5	31·0	300
20	750	+K	463	14·5	11·3	1·35	5·25	473	1·78	1·58	24·8	33·6	357
20	750	−K	491	14·6	2·0	2·33	5·13	377	2·15	1·47	21·0	34·9	328
75	350	+K	471	9·4	12·9	1·70	4·85	518	2·20	1·96	25·5	46·7	286
75	350	−K	475	12·6	2·2	2·44	5·22	385	2·13	1·81	17·8	34·1	300
75	750	+K	458	10·3	11·2	1·30	4·98	466	1·73	1·73	29·8	33·3	258
75	750	−K	487	15·3	1·9	2·14	3·05	335	1·99	1·18	13·0	39·9	244
			Effects										
O_3			*	***	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	*
CO_2			n.s.	n.s.	*	(*)	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
$O_3 \times CO_2$			n.s.	(*)	n.s.	n.s.	(*)	n.s.	n.s.	**	n.s.	n.s.	n.s.
Soil			***	**	***	***	n.s.	***	n.s.	**	**	n.s.	n.s.
Soil × O_3			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil × CO_2			**	***	*	(*)	n.s.	n.s.	**	n.s.	n.s.	**	n.s.
$O_3 \times CO_2 \times$ soil			n.s.	n.s.	n.s.	n.s.	(*)	n.s.	n.s.	(*)	n.s.	n.s.	n.s.
Clone			**	n.s.	n.s.	n.s.	(*)	***	n.s.	***	***	n.s.	**

Trees were grown in a native forest soil fertilized to achieve two levels of potassium nutrition; well fertilized (+K), potassium-deficient (−K). Data represent the mean of two clones (nos 773 and 399). Element concentrations: C, N, K, Mg, Ca, P (mg g^{-1} d. wt); S, Cu, Zn, Fe, Mn (µg g^{-1} d. wt).

Significance of main effects: n.s., not significant; *$P < 0{\cdot}1$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

concentrations of K, Mg, Ca, S, P, Zn and Fe (nor on Cu and Mn in 1988 needles), nor on the C/N ratio of foliage.

By contrast, CO_2 enrichment resulted in significantly lower concentrations of K and P (effects on Mg were also on the borderlines of statistical significance) in current-year needles, but there was no influence on the nutrient composition of the previous-year needles (although effects on N were on the borderlines of statistical significance). The C/N ratio of both current-year and previous-year needles was increased ($P < 0{\cdot}05$). There was little evidence of interactions between elevated CO_2 and O_3.

Throughfall chemistry

Figure 3 shows the effects of season-long exposure to elevated CO_2 and/or O_3 on the K, Mg and Ca contents of throughfall following successive simulated acid (pH 4·0) mist episodes. Significant differences in throughfall chemistry were found between clones; trees of clone 399 generally exhibiting higher rates of nutrient leaching. K-deficient trees exhibited *c.* 50 % lower levels of K in throughfall, but there were no significant effects on Mg and Ca concentrations. Responses to CO_2 and/or O_3 (and generally fertilization) were, however, generally the same so throughfall chemistry results are presented and discussed independently of clone and soil.

Initial misting events resulted in high concentrations of K, Mg and Ca in throughfall, but the rate at which nutrients were leached from foliage declined with subsequent mist episodes and attained a constant level after the 4th application. The actual losses of nutrients, calculated from misting volumes and leaching concentrations, represented less than 4 % of the needle contents. RM-ANOVA revealed that the throughfall collected from CO_2-enriched trees contained significantly higher concentrations (25–30 % averaged over 12 d misting period) of K ($P < 0{\cdot}05$), Mg ($P < 0{\cdot}05$) and Ca ($P < 0{\cdot}01$). By contrast, O_3 *per se* had no significant effect on the K, Mg and Ca contents of throughfall, and CO_2-induced increases in leaching were maintained in the presence of higher concentrations of O_3.

DISCUSSION

Only one chamber was used for each of the two carbon dioxide and ozone concentration treatments employed in this study. However, since the climatic conditions and gas concentrations in the treatment chambers were almost identical, and previous experiments had shown no significant differences in

Table 4. *Effects of a season-long exposure to elevated CO_2 (750 vs. 350 μmol mol^{-1}) and/or O_3 (75 vs. 20 nmol mol^{-1} 24 h d^{-1}), superimposed on an entirely artificial climate recreating that at a high elevation site in the Bavarian Forest, on the nutrient composition of previous (1988) year needles of Norway spruce* (Picea abies)

Treatment			Nutrient composition of 1988 needle year									
O_3	CO_2	Soil	C	N	K	Mg	Ca	S	P	Cu	Zn	Fe	Mn
20	350	+K	479	12·5	9·66	2·02	12·8	521	1·46	1·94	68·9	64·4	710
20	350	−K	490	14·7	1·71	2·75	12·4	423	1·33	1·94	38·9	78·5	710
20	750	+K	471	11·3	9·23	1·99	14·8	528	1·47	2·15	69·7	62·6	661
20	750	−K	494	13·8	1·78	2·67	13·4	433	1·39	2·26	55·7	63·5	637
75	350	+K	478	11·9	8·99	1·69	12·0	454	1·17	2·43	53·6	615	691
75	350	−K	472	13·8	1·76	2·87	14·8	442	1·29	2·23	50·4	85·7	779
75	750	+K	467	10·7	8·91	1·77	11·6	493	1·53	2·01	64·3	50·2	620
75	750	−K	490	12·9	1·62	2·70	13·2	401	1·37	1·91	52·6	64·4	738
			Effects										
O_3			*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CO_2			n.s.	(*)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$O_3 \times CO_2$			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
Soil			***	**	***	***	n.s.	***	n.s.	n.s.	*	n.s.	n.s.
Soil × O_3			n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil × CO_2			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$O_3 \times CO_2 \times$ soil			n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Clone			**	**	***	***	n.s.	***	*	***	***	(*)	n.s.

Trees were grown in a native forest soil fertilized to achieve two levels of potassium nutrition; well fertilized (+K), potassium-deficient (−K). Data represent the means of two clones (nos 773 and 399). Element concentrations: C, N, K, Mg, Ca, P, (mg g^{-1} d. wt); S, Cu, Zn, Fe, Mn (μg g^{-1} d. wt).

Significance of main effects: n.s., not significant; *$P < 0{\cdot}1$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

tree growth between chambers (Blank *et al.*, 1990), there seemed no reason to expect significant chamber-to-chamber variation other than with regard to the factors under investigation. This expectation was confirmed by routine measurements of light, temperature, humidity, CO_2 and O_3 in the individual chambers comprising the phytotron (Figs. 1, 2).

Soil, fine-root and foliar nutrient analyses indicated that contrasting fertilization programmes aimed at two levels of K nutrition achieved their goal. At the beginning of the experiment, in April 1989, K concentrations were 7–9 mg K g^{-1} needle d. wt for well fertilized trees and 4–5 mg K g^{-1} needle d. wt for trees grown in soil receiving no additional K fertilizer, a level comparable with that in healthy and chlorotic spruce needles in the Calcareous Bavarian Alps (Polle *et al.*, 1992) and regarded as approaching deficiency levels (Hüttl, 1985). Differences in foliar K intensified over the course of the season and although they were associated with a decline in the foliar concentrations of S, Zn, and also of Cu in the 1989 needles, K was the only nutrient whose level approached concentrations that would normally be regarded as limiting to the growth of Norway spruce (Hüttl, 1985). Foliar N concentrations were low for all trees (9–15 mg g^{-1} d. wt) but, as for the majority of elements, were similar to those reported for spruce in the Calcareous Bavarian Alps (see Pfirrmann *et al.*, 1990; Polle *et al.*, 1992). S and Cu levels were, however, somewhat lower than those reported for trees in the Bavarian Alps, whereas Mn levels were higher (see Polle *et al.*, 1992).

K availability is an important factor determining tree growth and vitality in certain parts of central Europe, but few controlled studies have addressed the effects of K deficiency *per se* on tree physiology (see Keller & Matyssek, 1990). In the present study, one of the most noticeable effects of K deficiency was the relative increase in the amount of K leached from needles. Although foliar K concentrations were reduced by five to sixfold in K-deficient trees, chemical analyses revealed a decrease of only 50 % in the amount of K appearing in throughfall. This observation is consistent with the reported effects of K deficiency on conifer needle cuticles (see Ylimarto *et al.*, 1994).

Soil analyses showed that exposure of trees to elevated CO_2 and/or O_3 resulted in subtle changes in the availability of certain elements in soil (Table 1). CO_2 enrichment, for example, was found to increase soil CECeff, an indicator of general soil condition (Meiwes *et al.*, 1984). The percolation of throughfall into the soil was prevented by a polythene cover

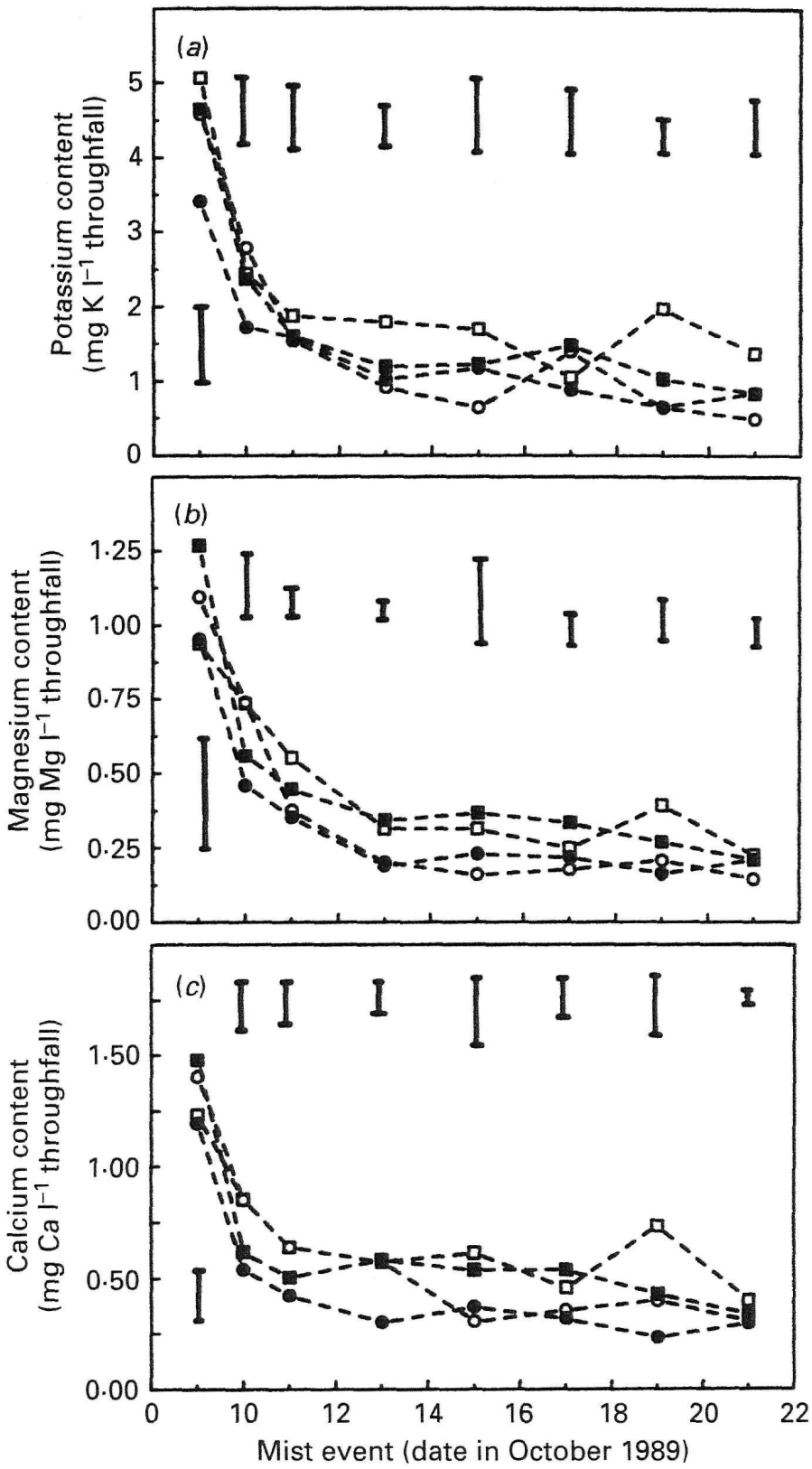

Figure 3. Effects of a seasonal exposure to elevated CO_2 and/or O_3 on the K, Mg and Ca concentrations in throughfall collected following a series of simulated acid mist (pH 4·0) events. Data presented is averaged over K treatments and clone, as these factors did not influence the response to elevated CO_2 and/or O_3. Ambient CO_2 (circles), elevated CO_2 (squares), 'non-polluted air' (open symbols), 'polluted air' (closed symbols). Vertical bars indicate the LSD calculated at the 5% level.

fitted to the surface of each pot, and direct effects of CO_2 enrichment on soil microbial activity would not be expected because rhizosphere CO_2 concentrations are naturally higher than 700 μmol mol^{-1} in any case (Scheffer & Schachtschabel, 1989), so the most likely explanation for the effect of elevated CO_2 on CECeff is an effect on soil microbial activity via the impact of elevated CO_2 on the above-soil parts of the tree. Microbial biomass/activity is known to be greater in the rhizosphere and bulk soil of plants grown at elevated CO_2 (Zak *et al.*, 1993) and this is highly contingent with the increased excretion of carbohydrates from the roots of plants growing under CO_2-enriched conditions (Norby *et al.*, 1987). We suggest therefore that the increased net photosynthetic carbon gain resulting from CO_2 enrichment (see Barnes *et al.*, 1995*a*) might have been reflected in a substantially increased rate of carbon input into the rhizosphere, which, combined with an increase in root size (Pfirrmann, 1992), stimulated microbial activity and increased CECeff. It remains to be established whether similar effects occur in the field in response to prolonged CO_2 enrichment because longer-term exposure to elevated CO_2 has been shown to increase the C/N ratio of litter through increases in carbon-based secondary compounds such as lignin and tannins (Fajer, Bowers & Bazzaz, 1992), and this might reduce decomposition and nitrogen mineralization processes, leading to a progressive immobilization of nutrients in the soil (Kirschbaum *et al.*, 1994).

Elevated CO_2 reduced the K and P (and effects on Mg were on the borderlines of statistical significance) contents of current-year needles, but had no significant influence on the nutrient composition of the previous-year needles (although effects on N were on the borderline of statistical significance). This implies that uptake and transfer of these nutrients into the current-year needles did not satisfy the demand generated by enhanced growth at elevated CO_2 (see Norby, O'Neill & Luxmore, 1986; O'Neill, Luxmore & Norby, 1987; Polle *et al.*, 1993). However, care needs to be taken in the interpretation of these data because starch accumulated in the needles of CO_2-enriched trees over the course of the season (Barnes *et al.*, 1995*a*). Losses of K, Mg and Ca in throughfall were of the same low order of magnitude as observed during previous experiments (Pfirrmann *et al.*, 1990), but cannot be neglected. One factor contributing to the decline in the K and Mg contents of the current-year needles of CO_2-enriched trees appeared to be the elevated rate at which these elements were leached from the foliage of trees exposed to elevated CO_2 (Fig. 3). The reason for this is unclear. However, recent work has shown that CO_2 enrichment can induce marked changes in the chemical composition of the waxes on the surface of spruce needles (Prügel, 1994) and this would be expected to cause changes in needle wettability, a factor known to affect the rate at which cations are leached from foliage (see Wellburn *et al.*, 1996). The observation that elevated CO_2 may increase the rate at which cations, and possibly other solutes, are leached from foliage has far-reaching implications, and the mechanisms underlying these effects are worthy of further investigation. Decreased foliar concentrations of N, P, K, Ca and Mg have commonly been reported in trees, and also in herbaceous species, in response to CO_2 enrichment, but enhanced leaching of nutrients from foliage has not previously been considered as a contributing factor (see Oberbauer *et al.*, 1986; Brown, 1991).

Over the past decade, considerable attention has been paid to the effects of O_3 on Norway spruce

because of suggestions that the pollutant contributes to the development of the regional-specific nutrient disorders characteristic of particular types of forest decline in mountainous areas of central Europe (see Roberts *et al.*, 1989). However, in recent years the role of O_3 (Sandermann, Wellburn & Heath, 1996), and even the very existence of a widespread form of 'novel' forest decline, has been increasingly questioned (Skelly & Innes, 1994). In the present study, exposure to a seasonal O_3 regime realistic for high-altitude areas of the Bavarian Forest resulted in few significant changes in foliar nutrient composition and there was no evidence that O_3 influenced the rate at which K, Mg and Ca were leached from foliage. These observations lend further support to the argument that reports in the mid-1980s of increased foliar leaching induced by 'ozone' resulted from contamination of the O_3 used to fumigate trees (see Brown & Roberts, 1988). The present study was restricted to a single growing season, so longer-term effects cannot be discounted (Lucas, Rantanen & Mehlhorn, 1993). However, given the present findings, a mechanism for such longer-term effects is difficult to reconcile and it is noteworthy that a number of experiments on Norway spruce conducted over several seasons have revealed no significant effects of long-term O_3 exposure on foliar nutrient composition (Roberts *et al.*, 1989; Pfirrmann *et al.*, 1990; Ogner, 1993; Rantanen, Palomäki & Holopainen, 1994). The present study therefore lends further support to the growing scepticism concerning the role of O_3 in the development of Mg and K deficiency symptoms typical of 'declining' trees at higher elevations in the German 'Mittelgebirge' and Bavarian Forest.

Research into the combined effects of elevated CO_2 and O_3 on plant growth, physiology and biochemistry is still at an early stage (see Barnes & Pfirrmann, 1992; Van der Eerden *et al.*, 1993; Barnes, Ollerenshaw & Whitfield, 1995*b*; Barnes *et al.*, 1995*a*; Balaguer *et al.*, 1995). There is a widely held opinion that CO_2-induced decreases in stomatal conductance will afford additional protection against O_3 damage (Allen, 1990). However, recent experiments on wheat (Barnes *et al.*, 1995*b*), spruce (Barnes *et al.*, 1995*a*), birch (Mortensen, 1995) and clover (Van der Eerden *et al.*, 1993) have revealed that although the growth of O_3-treated plants may be stimulated by higher CO_2, the relative impact of O_3 is commonly the same at ambient and elevated CO_2, despite additive effects of the gases on stomatal conductance in some cases. The lack of significant $CO_2 \times O_3$ interactions in the present study was consistent with previous observations, since the combined effects of the gases on nutrient supply, content and throughfall chemistry were no more or less than additive.

Given that large-scale models of regional and global vegetation dynamics require parameterization with data consistent with changes in plants under natural conditions, it is clearly important to undertake larger-scale *in situ* experiments on mature woody species. However, there is an immediate need to improve our understanding of the way in which the impact of elevated atmospheric concentrations of CO_2 on tree growth and physiology will be influenced by other environmental factors (e.g. gaseous air pollutants, nutrient limitations, pests and pathogens, soil-moisture deficit and winter stress). At the present time this information can only be obtained realistically in smaller-scale controlled-environment studies, although there is a clear need for longer-term studies to be conducted in the field. Further work is urgently required to improve our present knowledge of plant, and in particular tree, responses to the combination of rising CO_2 and O_3 (Garrec, Laitat & Rose, 1993). It is important that we understand how plants will respond to this combination of gases, not only to define the mechanisms involved in the modification of plant responses to complex changes in climate and atmosphere, but also to permit the correct interpretation of plant responses to elevated CO_2 and mixtures of pollutants in studies employing controlled-environment chambers, open-top chambers and open-field exposure systems.

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