Plant, Cell and Environment (2002) **25,** 501–512

Nitrogen dynamics during O_3-induced accelerated senescence in hybrid poplar

D. G. BIELENBERG,[1] J. P. LYNCH[2] & E. J. PELL[3]

[1]*Intercollege Graduate Program in Plant Physiology, The Pennsylvania State University, 301 Buckhout Laboratory, University Park, PA 16802, USA,* [2]*Department of Horticulture, The Pennsylvania State University, 221 Tyson Building, University Park, PA 16802, USA and* [3]*Department of Plant Pathology and Environmental Resources Research Institute, The Pennsylvania State University, 304 Old Main, University Park, PA 16802, USA*

ABSTRACT

Experiments were conducted to determine the fate of nitrogen (N) remobilized as a result of ozone (O_3)-induced accelerated senescence in hybrid poplar subjected to declining N availability concurrent with O_3 stress. Cuttings were grown in sand culture where the supply of N to the plant could be controlled on a daily basis and reduced in half of the plants when desired. Plants all initially received 3·57 mM N daily until approximately the 20 leaf stage after which daily supply of N was reduced to 0·71 mM. Plants were grown in open-top chambers in the field (Rock Springs, PA, USA) and received charcoal-filtered air, half also received supplemental O_3 to a level of 0·08 μL L^{-1}. Allocation of newly acquired N was determined with ^{15}N. The specific allocation (mg labelled N mg^{-1} total N) of labelled N to upper, expanding leaf N was not affected by O_3, but was strongly affected by N treatment. However, O_3 increased the relative partitioning of labelled N to the expanding leaves and the roots. The balance between partitioning of newly acquired N to the upper leaves and roots was not affected by O_3, but was reduced by N withdrawal. Calculated net N flux was strongly negative in the lower leaves of O_3-exposed, N withdrawal plants. Nitrogen uptake was not reduced by O_3. The allometric relationships between the roots and shoots were not affected by O_3 or N availability. The relative contribution of newly acquired versus remobilized N to new growth appears to be determined by N supply. Ozone exposure alters the allocation of newly acquired N via alterations in plant size, whereas N availability exerts a strong effect upon both plant size and N allocation.

Key-words: accelerated foliar senescence; allocation; ^{15}N; nitrogen; ozone; partitioning; redistribution; uptake.

INTRODUCTION

The air pollutant O_3 is the primary phytotoxic component of photochemical smog and is responsible for reductions in growth and yield of vegetation in natural and managed landscapes around the world (Kickert & Krupa 1990; Krupa 1997). Ozone can reduce growth by accelerating the normal rate of foliar senescence (Reich & Lassoie 1985). Leaves exposed to O_3 show an accelerated progression of the hallmarks associated with senescence. These include declines in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), declines in net photosynthesis, increased proteolysis, altered fine organelle structure and increased membrane leakage (Ojanperä *et al*. 1992; Pääkkönen, Holopainen & Kärenlampi 1995; Sandelius *et al*. 1995), and culminate in early abscission and leaf loss. Leaf exposure to O_3 has been associated with accelerated loss of quantity and activity of Rubisco, the primary carboxylating enzyme of photosynthesis (Pell & Dann 1991). This decrease in quantity was determined to be the result of increased proteolysis and not decreased synthesis (Brendley & Pell 1998). Loss of Rubisco is associated with reductions in individual leaf net photosynthesis and, potentially, whole plant carbon (C) gain (Greitner, Pell & Winner 1994).

The rate at which leaf senescence progresses is sensitive to a number of external environmental factors (Noodén & Leopold 1988), including soil N level. It is known that the N supply during plant growth influences the degree of O_3-induced accelerated foliar senescence (Pell, Sinn & Vinten-Johansen 1995; Pääkkönen & Holopainen 1995). It has also been shown that fluctuations in the N available during the growing season influences O_3-induced accelerated senescence. Bielenberg, Lynch & Pell (2001) recently reported that the degree of leaf abscission in response to O_3 exposure was markedly increased by a decline in the concentration of N supplied to the plants on a daily basis. Plant response to dynamic N supply is of particular significance because plants frequently receive the large doses of N early in the growing season, ironically when they are experiencing the least demand. In temperate ecosystems N levels can be highest in the spring when conditions support release of nutrients from litter that may have been deposited the previous autumn (Haynes 1986).

Ozone-induced accelerated senescence may support new leaf growth if nutrient resources are remobilized from older, senescing foliage. When older leaves of O_3-exposed plants experience a more rapid rate of senescence, the younger tissues of the same plants have been shown to demonstrate increased net carbon assimilation (Pell *et al*.

Correspondence: Dr Eva J. Pell, Steimer Professor of Agricultural Sciences, The Pennsylvania State University, 304 Old Main, University Park, PA 16802-1504, USA. Fax: 814-863-9659, e-mail: ejp@psu.edu

© 2002 Blackwell Science Ltd

1994), synthesis and quantity of Rubisco (Brendley & Pell 1998), nitrogen content and total soluble protein (Temple & Riechers 1995), and amino acid concentrations (Manderscheid, Jager & Kress 1992) relative to comparable young tissues in charcoal-filtered plants. It has been suggested that the increased C gain in younger leaves is compensatory, and may help to offset reduced C gain in older tissues (Pell *et al*. 1994). Resources mobilized during O_3-induced accelerated senescence may be translocated elsewhere in the plant (Manderscheid *et al*. 1992; Temple & Riechers 1995), in order to compensate for stress effects on older leaves. When the effect of declines in N availability on O_3-induced accelerated senescence was investigated (Bielenberg *et al*. 2001), compensatory responses of young leaves to O_3 exposure only occurred when N availability to the plant declined and O_3-induced accelerated senescence was most severe.

The basis of the influence of N availability upon O_3-induced accelerated senescence and the link to compensatory physiology is not known. Ozone and N availability may interact at the level of N acquisition from the soil. Obviously, reduced N availability in the soil will reduce N uptake by the plant. Few studies have assessed N uptake by O_3-exposed plants; it has been shown that O_3 may negatively impact N uptake by the roots (Nouchi *et al*. 1991; Pausch *et al*. 1996b). Other studies have shown reduced respiration by root systems as a result of O_3 exposure, which could affect nutrient acquisition (Edwards 1991). Reduced N availability may increase partitioning of N to the roots (Marschner 1995). The remobilization of nutrients from senescing leaves may provide an increasingly important source of usable N should reduced N uptake fail to meet demand for new growth. Ozone-induced accelerated senescence of older foliage could influence the movement and destination of N within the plant. Senescence of older leaves could alter allocation of N to leaf parts by eliminating sinks for xylem-delivered N thereby enhancing N delivery to the remaining leaves (Jordi *et al*. 2000).

It has been proposed that plants exhibit shifts in biomass allocation to those structures that are responsible for the acquisition of a limiting resource such as light or nitrogen (Brouwer 1962). Greater biomass allocation to shoots relative to the roots is often observed in O_3-stressed plants (Cooley & Manning 1987). A number of studies with isotopic tracers have shown that proportionally less photosynthate is translocated from leaves to roots in O_3-stressed plants (Nouchi *et al*. 1995; Samuelson & Kelly 1996; Pausch *et al*. 1996a). These changes in translocation are generally ascribed to increases in foliar respiration or alterations in carbohydrate partitioning resulting in reduced export (Cooley & Manning 1987). Decreased translocation of C to the roots is viewed as the cause of decreased root mass in O_3-stressed plants. In contrast, N limitation promotes the growth of roots at the expense of the shoot (Gutschick & Kay 1995; van der Werf & Nagel 1996; Marschner, Kirkby & Cakmak 1996). Therefore, O_3 and N may interact antagonistically, imposing conflicting priorities for biomass allocation between the roots and the shoots.

The objective of this study was to determine the nature of the interaction between O_3 and N availability as it relates to O_3-induced accelerated senescence of older foliage and the expression of compensatory physiology by young foliage. In order to address this objective two hypotheses were formulated. First, N remobilized during O_3-induced accelerated senescence is incorporated into young leaves. Secondly, O_3 exacerbates a decline in N available to the plant by decreasing C allocation to root biomass. These hypotheses were tested by growing a hybrid poplar clone known to demonstrate O_3-induced accelerated senescence in a sand culture system which allowed control of N availability and the introduction of labelled N with no disturbance to the plant.

MATERIALS AND METHODS

Plant material

Hybrid poplar (*Populus trichocarpa* × *maximowizii* clone '245') cuttings were used in all experiments. Cuttings were obtained in February or March for use in each year's experiment from a stand at the Russell E. Larson Agricultural Research Farm at Rock Springs, Pennsylvania, USA. Cuttings were preserved in dormancy at 4 °C in a cold room until planting. During the experiment, all side shoots were trimmed from the plant at initiation so that the shoot consisted of a single axis of growth.

Nutrient regimes

Cuttings were planted in 9 L pots containing unsifted sand derived from crushed quartz (Beavertown Block Co., Pleasant Gap, PA, USA). The cuttings were planted on day of year 155, 153 and 154 in 1996, 1997 and 1998, respectively. For reference, day of year 153 is the equivalent of 1 June in 1996 and the equivalent of 2 June in 1997 and 1998. All ramets were watered daily with a solution containing a complete nutrient formula, pH = 5·7 ± 0·1. The containers had drainage holes and a liquid-holding capacity of approximately 3–4 L of nutrient solution. Nutrient solution was supplied via the irrigation lines to the plant; two to three times the holding capacity of the pots was supplied daily to ensure flushing of old nutrient solution from the pots and replacement by full-strength solution. At no time was water limiting to the plants. Each year by the end of the experiment the root systems of the plants had fully explored the volume of the pots, but no cessation of root growth was observed to indicate a pot-bound condition. All plants initially received 3·57 mM N daily. After 25, 30 and 36 d of O_3 exposure in years 1996, 1997 and 1998, respectively, half of the chambers in the charcoal-filtered and O_3-added treatments began receiving 0·71 mM N daily.

The nutrient solution contained: N (3·57 mM, 3 : 1 NO_3 : NH_4); P (0·92 mM); K (2·69 mM); Mg (1·0 mM); Ca (1·1 mM); S (1·0 mM); Fe (9·474 μM); Zn (4·848 μM); Cu (2·55 μM); Mn (10·26 μM); B (8·806 μM); Mo (0·0292 μM). Nitrogen was supplied as a combination of NH_4NO_3 and KNO_3. The N

withdrawal treatments contained 0·71 mM N obtained by reducing the NH_4NO_3 and KNO_3 by 80%. The K^+ lost from the reduced level of KNO_3 was replaced with K_2SO_4 to achieve the same final concentration of K^+.

Air treatments

Plants were grown in 12 (1996, three chambers per treatment combination) or 16 (1997 and 1998, four chambers per treatment combination) open top chambers equipped with rain exclusion caps (Brendley & Pell 1998). Ozone was generated from pure O_2 and was dispensed and monitored as previously described (Pell *et al*. 1993). The air entering all chambers was charcoal-filtered throughout the 24 h with half of the chambers receiving a supplement of 0·08 μL L^{-1} O_3 from 1000 to 1800 h daily. The O_3 treatments began 23, 17 and 16 d after planting in the 1996, 1997 and 1998 experiments, respectively, after buds had broken on the cuttings and the stems had begun to elongate. Photosynthetic photon flux density, relative humidity and air temperature within the chambers were monitored as described by Pell *et al*. (1993).

Biomass

Six times during the 1996 experiment one plant was randomly selected from each chamber to be destructively harvested. The plant was divided into five fractions for biomass and N determination: upper (expanding) leaves, middle leaves, lower leaves, stems and roots. The leaf positions (marked by the leaf scar in case of abscission) not included in the upper leaf category were evenly divided between middle and lower leaf fractions. Petioles were included in the leaf fractions. Plant fractions were dried at 70 °C for 7 d for biomass determination.

Destructive harvests were conducted in the same manner in 1997 and 1998 with the exception that nine harvests were conducted in 1998. Also, petioles were included with the stem fraction rather than with the respective leaf fractions in these years.

Total nitrogen determination

In the 1996 and 1997 experiment the total N was determined in the dried plant fractions by an EA 1108 elemental analyzer operating in CHN mode using acetinilide as a standard (Fisons Instruments, Milan, Italy). Prior to analysis the samples were dried and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) through a 40 mesh screen. Plant fractions from the 1998 experiment were analysed for total N content by micro-Dumas combustion analysis at the Institute of Ecology's Stable Isotope/Soil Biology Laboratory at the University of Georgia, Athens, GA, USA.

^{15}N tracer technique

Plants were given a pulsed dose of ^{15}N-labelled nutrient solution in the 1998 experiment. The pulse was given 2 d after the decrease of N in the withdrawal treatment and prior to significant leaf abscission. The containers of plants to receive the labelled nitrogen were flushed thoroughly with water the evening before the pulse was applied. All fallen leaves and other debris were removed from the containers prior to the application of the ^{15}N. The labelled N was applied as a nutrient solution identical to the solution the plants received daily with the exception that the nitrogen in the solution was 10 atom% ^{15}N. The day after the ^{15}N application the containers were again flushed to remove the labelled nutrient solution. The plants were harvested 8 d after the day of ^{15}N application to assess distribution of the labelled N among the plant fractions. Samples were sent to the Institute of Ecology's Stable Isotope/Soil Biology Laboratory at the University of Georgia (Athens, GA, USA) where the samples were dried, ball-milled and analysed for ^{15}N : ^{14}N via mass spectrometry in series with elemental analysis to determine total N content. It was assumed that every atom of ^{15}N taken up by the plant represented nine additional atoms of ^{14}N because the N source made available to the plant was 10% ^{15}N in composition. The term 'new N' was used to denote the total amount of N (^{15}N and ^{14}N) newly acquired by the plant.

Allocation of newly acquired N was expressed using two parameters: specific allocation to a plant tissue of newly acquired N [mg N (new) mg^{-1} N (total) in a particular plant tissue] and relative partitioning of newly acquired N among plant tissues [mg N (new) in a plant tissue mg^{-1} N (new) in the whole plant].

Data analysis and statistics

Separate from the ^{15}N experiments, N flux (mg N d^{-1}) was calculated by numerical differentiation of the N content determined from destructive harvests of each organ (Lynch & White 1992), using least squares fitting of quadratic polynomials by a three-point formula (Erickson 1976).

The experimental design was a 2×2 factorial design (two N treatments and two O_3 levels) replicated three (1996) or four (1997 and 1998) times resulting in a total of 12 (1996) and 16 (1997 and 1998) chambers, respectively. Treatment combinations were assigned to chambers and the chambers randomized in the field. When necessary, data were normalized by transformations. All plant dry weight (DW) and N content values were $\log_e$ transformed to normalize the data and allow for the calculation of the relative growth rate (RGR) of the parameter, namely the slope of the $\log_e$ dry weight or N content versus time (units: g g^{-1} d^{-1} or g N g^{-1} N d^{-1}). The RGR is an index of efficiency of growth per unit plant tissue and allows direct comparison of the performance of plants or plant parts of varying size.

Treatment effects on variables were evaluated using ANOVA with N, O_3 and date of harvest as class variables. Data were analysed by repeated measures with date as the repeated factor and chamber as the subject. A first-order autoregressive covariance structure was used to adjust for correlations between adjacent harvest dates. The mean square associated with the $O_3 \times N$ interaction nested within

replicate was used to test significance of the main effects and interactions. The residual error term was used to test significance of date alone and interaction with the treatment effects. Analyses were performed for each year separately, using the Mixed Models procedure of SAS (SAS Institute, Inc., Cary, NC, USA). Only harvest dates that occurred after the N withdrawal were analysed.

The allometric coefficient K, representing the ratio between the RGR of the shoot and the RGR of the roots was determined from the slope of the relationship between the $\log_e$ g dry weight of the shoot and the $\log_e$ g dry weight of the roots. Relative growth rates of biomass (g g^{-1} d^{-1}) and N content (g N g^{-1} N d^{-1}) were calculated from the slope of the relationship between $\log_e$ g dry weight or $\log_e$ mg N and harvest date. Tests of significance for differences among slopes (K or RGR) were performed using the regression procedure of SAS.

RESULTS

Allocation of newly acquired N

Nitrogen withdrawal significantly reduced the specific allocation (mg new N per mg total N) of newly acquired N to the N pool in upper, expanding leaves (Fig. 1, Table 1). Ozone did not significantly change the specific allocation of new N to the upper, expanding leaves in either of the N treatments (Fig. 1, Table 1).

A greater proportion of the newly acquired N (relative partitioning, mg new N plant part per mg new N whole plant) was delivered to the upper, expanding leaves in response to O_3 exposure in both N treatments (Fig. 2, Table 1). Within air treatments, the constant N treatment allocated a greater share of the new N to the upper, expanding leaves (Fig. 2, Table 1). Ozone and N both appeared to increase the relative partitioning of new N to the roots (Fig. 2). However, these trends were not statistically significant (Table 1).

The relative strength of the upper leaves and roots as a sink for newly acquired N was assessed by the ratio of relative partitioning of newly acquired N to upper leaves to relative partitioning of newly acquired N of the roots. When corrected for whole plant biomass, only N treatment exerted a statistically significant effect upon the ratio of N allocation, with N withdrawal reducing N allocation to young leaves relative to the roots (Fig. 3, Table 1).

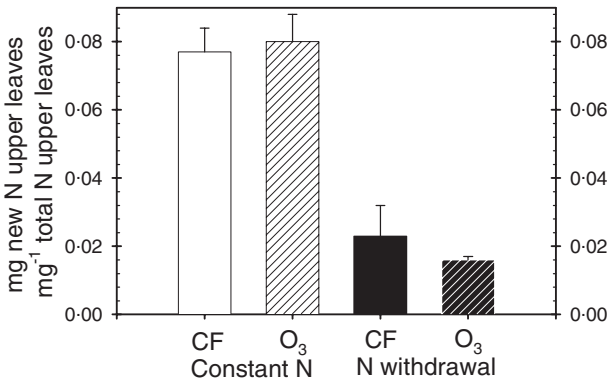

Figure 1. Specific allocation to upper, expanding leaves of newly acquired N that originated from a 24 h pulse of ^{15}N-labelled nutrient solution (10% ^{15}N) given to hybrid poplars 8 d earlier. Plants were grown in sand in open-top chambers receiving charcoal-filtered (CF) air or charcoal-filtered plus supplemental O_3 to a concentration of 0·08 μL L^{-1} (O_3). Constant N plants received 3·57 mM N daily for the entire experiment. N withdrawal plants received 3·57 mM N daily for 3–4 weeks of O_3 exposure after which N concentration was reduced to 0·71 mM N daily. Values represent the mean of four replicates ± SE.

Biomass and N content of whole plant and plant parts

The biomass and N flux experiments were repeated in 1996 and 1997. The relative responses between the treatment combinations were similar in both experiments. Therefore, only data from 1997 were chosen for graphic presentation (Figs 4–7).

Biomass and N content of the whole plant and plant fractions was determined by destructive harvests over a period of time in the 1997 experiment and this relationship was plotted on a semi-log ($\log_e$ g DW or $\log_e$ mg N versus

Table 1. Results of statistical analyses for the effects of air (O_3) and N treatment (N) upon labelled N distribution parameters in the 1998 experiment

N distribution parameter		(mg new N upper leaves mg^{-1} total N upper leaves)		(mg new N upper leaves mg^{-1} new N whole plant)		Relative partitioning to roots(mg new N roots mg^{-1} new N whole plant)		mg new N upper leaves mg^{-1} new N roots g^{-1} whole plant DW	
Source of variance	d.f.	F	$P > F$	F	$P > F$	F	$P > F$	F	$P > F$
N	1	66·57	**0·0001**	10·37	**0·0073**	3·52	0·0850	4·74	**0·0502**
O_3	1	0·08	0·7822	5·45	**0·0378**	2·73	0·1245	0·19	0·6719
N × O_3	1	0·31	0·5867	0·48	05016	0·69	0·4227	0·69	0·4227

Bold P-values are ≤ 0·05.

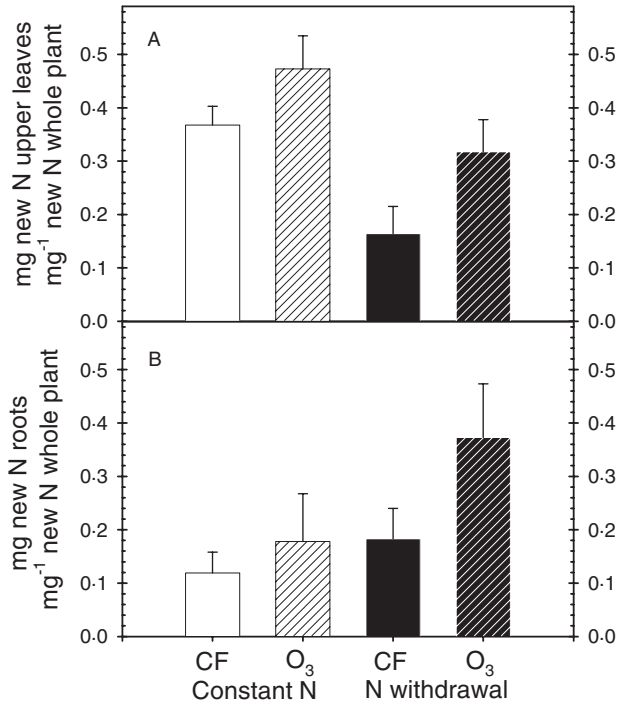

Figure 2. Relative partitioning of newly acquired N to the upper, expanding leaves (A) or the roots (B) that originated from a 24 h pulse of a labelled N solution taken up by plants on Julian day 208. Treatments are as in Fig. 1. Values represent the mean of four replicates ± SE.

day) scale (Figs 4 & 5, respectively). All measurements of biomass and N content increased with time. Responses on a $\log_e$ scale tended to be linear throughout the four harvest dates following the N withdrawal (Days 205, 219, 226 and 233). The lessening of increase between day 233 and day 240 was probably related to the cessation of growth associated with bud set.

The whole plant, upper leaves, stem and roots followed the same pattern of biomass increase with time (Fig. 4A, B, E & F). Biomass of the O_3-exposed plants was always lower than that of the charcoal-filtered controls, but neither the slopes of the O_3-exposed, constant N treatment or the N withdrawal treatments were significantly different from the charcoal-filtered, constant N control, despite a trend toward shallower slopes in the N withdrawal treatments (Fig. 4A, B, E & F). Biomass response of the middle leaf fraction was similar to that seen in the whole plant, with the exception that the charcoal-filtered, N withdrawal treatment had a significantly shallower slope than the charcoal-filtered, constant N treatment (Fig. 4C). Lower leaf biomass accumulation was lower in O_3-treated plants, and the rate of increase with time was reduced relative to the charcoal-filtered control in each of the N treatments (Fig. 4D). The biomass of lower leaves of plants exposed to O_3 and subjected to N withdrawal had a slightly negative slope with time; these leaves had abscised in several of the plants harvested at later dates in the experiment (Fig. 4D).

The pattern of N content with time was similar among the whole plant, middle leaves, stem and roots (Fig. 5A, C, E & F). The nitrogen content of the O_3-exposed plants was always lower than that of the charcoal-filtered controls; the slopes of the N content of the N withdrawal plants were significantly lower than the charcoal-filtered, constant N control (Fig. 5A, C, E & F). The upper leaf fraction was similar with the exception that the plants subjected to the N withdrawal had lower initial N content than the constant N treatments. The slopes of N content from the N withdrawal treatments were also shallower, but the slopes were not significantly different from the charcoal-filtered, constant N control (Fig. 5B). Lower leaf N accumulation was reduced by O_3 exposure and the rate of increase with time was reduced relative to the charcoal-filtered control in each of the N treatments (Fig. 5D). The plants exposed to O_3 and subjected to N withdrawal had a negative slope with time for the lower leaves, these leaves had abscised in several of the plants harvested at later dates (Fig. 5D).

N flux to plant parts

The net N flux (mg N d^{-1}) into the plant fractions (Fig. 6) was determined to evaluate whether treatment-induced differences in net N movement out of older, senescing leaves and into young, expanding leaves could be observed without the use of labelled N. Few statistically significant relationships were found in 1996 despite similar patterns of response to those seen in 1997 (Table 2). This is probably due to the smaller number of replicates used in 1996 (three in 1996 versus four in 1997) and the greater variability of that data set.

Relative N flux (g N g^{-1} N d^{-1}) was calculated for plant biomass fractions in the 1996 and 1997 experiment and statistically analysed for the middle four harvest dates (Days 205, 219, 226 and 233) where logarithmic rates of increase in biomass (relative growth rate, RGR) and N content (relative specific growth rate of N) were seen to be linear (Figs 4 & 5).

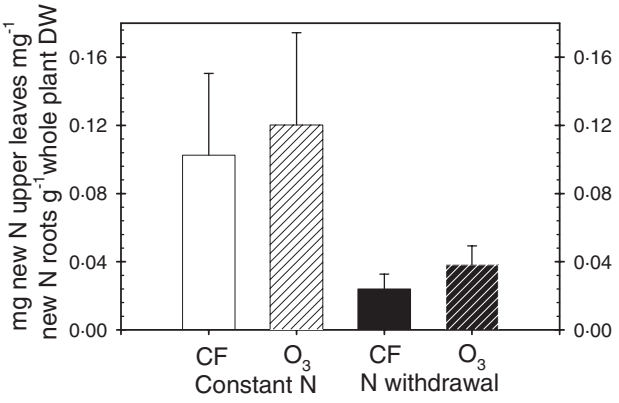

Figure 3. Ratio between relative partitioning of newly acquired N to upper, expanding leaves and to roots, corrected for whole plant biomass. Treatments are as in Fig. 1. Values represent the mean of four replicates ± SE.

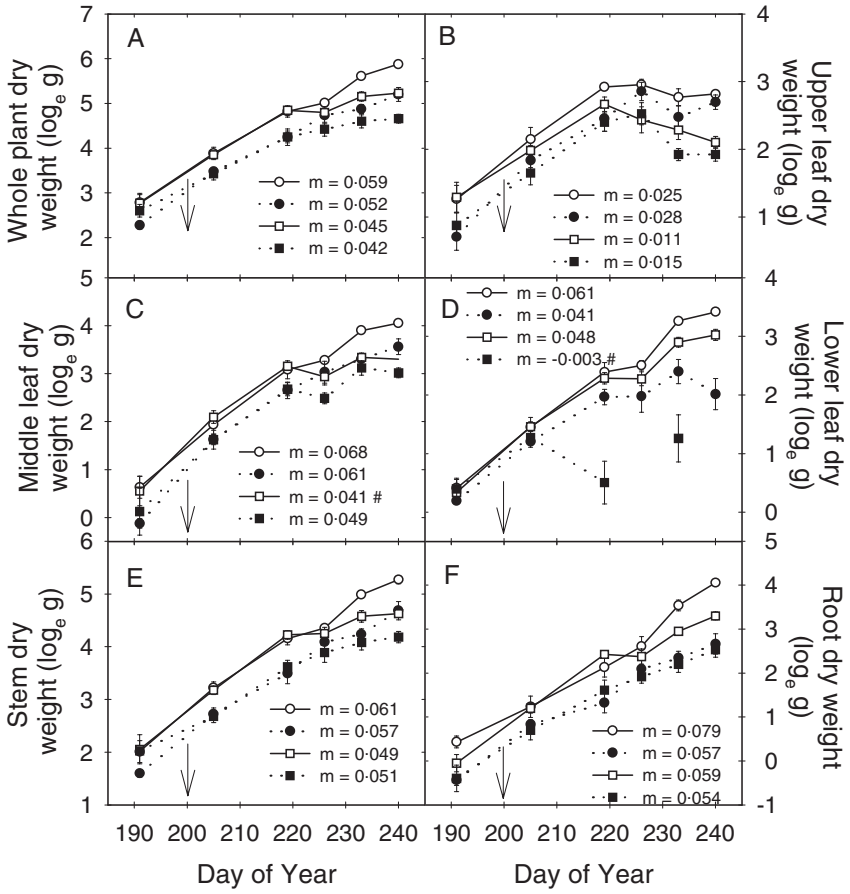

Figure 4. Biomass (log$_e$ g DW) of whole plants (A), upper leaves (B), middle leaves (C), lower leaves (D), stems (E), or roots (F) of hybrid poplar plants grown in open-top chambers receiving charcoal-filtered air (open symbols, solid lines) or charcoal-filtered plus supplemental O_3 to a concentration of 0·08 μL L^{-1} (closed symbols, dotted lines) in the 1997 experiment. Plants were grown in sand with nutrient solution provided daily. Plants received 3·57 mM N daily for the entire experiment (circles) or a 3·57 mM N daily for approx. 4 weeks of O_3 exposure after which N concentration was reduced to 0·71 mM daily (squares). Arrows indicate time of nutrient withdrawal. Values represent the mean of four replicates ± SE. Legend indicates slope (m; g g^{-1} d^{-1}) of time versus biomass relationship through the four middle harvests; a '#' symbol following a value indicates that it is significantly different ($P \leq 0·05$) from the charcoal-filtered, constant N control (open circles).

In both years the net flux of N into the upper, expanding leaves significantly decreased with time in all treatments, eventually becoming negative (1997) or zero (1996) by the end of the experiment (Table 2). Also, in 1997, N withdrawal significantly reduced net N flux into upper leaves (Fig. 6A & B, Table 2). The similar net N flux in charcoal-filtered and O_3-treated plants occurred despite slightly reduced biomass in the upper leaves of O_3-treated plants (Fig. 4B), indicating increased N concentration in response to O_3 exposure in both of the N treatments. Ozone exposure slightly increased the RGR of biomass in the upper leaves (0·025 versus 0028 g g^{-1} d^{-1} for constant N and 0·011 versus 0·015 g g^{-1} d^{-1} for N withdrawal, Fig. 4B), whereas in the same leaves the relative specific rate of growth of N was greatly accentuated (0·011 versus 0·022 g N g^{-1} N d^{-1} for constant N and –0·007 versus 0·007 g N g^{-1} N d^{-1} for N withdrawal, Fig. 5B).

Nitrogen withdrawal significantly reduced net N flux into the middle leaves in 1996 (Table 2). In 1997, N withdrawal reduced net N flux into middle leaves on the last two harvests and O_3 reduced net N flux in the constant N treatment to a greater degree than in the N withdrawal treatment (Fig. 6C & D). As a result, the N × date and N × O_3 interactions were significant (Table 2).

Similar trends were seen in both years for the net N flux into lower leaves. In the charcoal-filtered, constant N treatment the net N flux into lower leaves increased with time. Ozone exposure eliminated this increase in net N flux in the constant N treatment (Fig. 6E). In the N withdrawal treatment both air treatments had low but constant fluxes until midway through the experiment when O_3 reduced net N flux to zero or negative values. In 1997, lower leaf net N flux had a significant N × O_3 × date interaction (Table 2). This resulted from the strong negative net flux in the O_3-exposed, N withdrawal treatment (Fig. 6F), becoming more negative with time, and eventually ceasing when all leaves were lost from that segment. There were no significant treatment effects upon lower leaf net N flux in 1996 (Table 2). The lower leaves showed large differences in net N flux among treatments in (Fig. 6E & F). For the charcoal-filtered treatments, N withdrawal decreased the relative rate of N and biomass accumulation in lower leaves (Figs 5D & 4D) in comparison with plants grown at constant N. Reduced net N flux into the lower leaves of the plants treated with O_3 and grown with constant N relative to the charcoal-filtered control plants (Fig. 6E) occurred as a result of O_3-induced declines in N and biomass; the latter largely being the result of leaf loss (Figs 4D & 5dD). Net N flux into the lower leaves of plants treated with O_3 and subjected to N withdrawal became sharply negative (Fig. 6F) as a result of O_3-induced loss of biomass and N, eventually returning to zero net flux when all of the leaves had

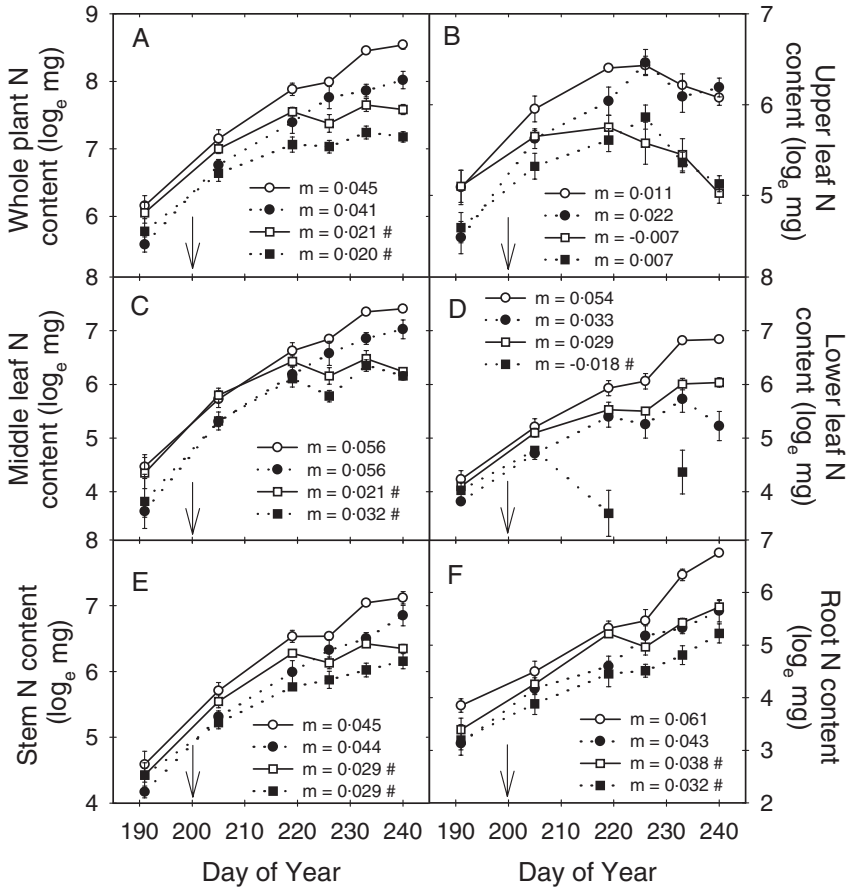

Figure 5. Nitrogen content (log$_e$ mg N) of whole plants (A), upper leaves (B), middle leaves (C), lower leaves (D), stems (E), or roots (F) of hybrid poplar plants in the 1997 experiment. Treatments and symbols are as in Fig. 4. Values represent the mean of four replicates ± SE. Legend indicates slope (m; mg N mg^{-1} N d^{-1}) of time versus N content relationship through the four middle harvests; a '#' symbol following a value indicates that it is significantly different ($P \leq 0{\cdot}05$) from the charcoal-filtered, constant N control (open circles).

abscised. The relative specific growth rate of N content with time (g N g^{-1} N d^{-1}) for the lower leaves of plants exposed to O_3 and subjected to N withdrawal was more strongly negative (–0·018 g N g^{-1} N d^{-1}, Fig. 5D) than that of biomass (–0·003 g g^{-1} d^{-1}, Fig. 4D), indicating that there was an accelerated loss of N. Therefore the lower leaves of plants in this treatment did appear to be returning N to the whole plant as a result of O_3-induced accelerated senescence.

The relative treatment response of stem and root net N flux to N withdrawal and O_3 was similar in both years. The main effect of O_3 and the N × date interaction were significant for net N flux into the stem in 1997 (Table 2). In 1996 no effects upon stem net N flux were significant (Table 2). The net N flux to the roots was significantly reduced by N withdrawal in 1996 (Table 2). In 1997 the N × O_3 × date interaction was significant for net N flux in roots (Table 2).

N uptake

Whole plant N uptake (mg N g^{-1} DW d^{-1}) directly measured by single day labelled N uptake in the 1998 experiment on day 208 was significantly reduced by N withdrawal and not affected by O_3 exposure (Table 3). Whole plant N uptake was also calculated from N content at several destructive harvests throughout the experiment in 1996 and 1997. Whole plant net N flux (equivalent to uptake; mg N d^{-1}) was significantly reduced by N withdrawal in 1996 (Table 4). Both N withdrawal and O_3 reduced N uptake in 1997; however, the effect of O_3 was greatest in the constant N treatment resulting in a significant N × O_3 interaction (Fig. 7, Table 4).

Root and shoot partitioning

For each treatment the allometric relationship of biomass partitioning between roots and shoots of hybrid poplar in the 1996, 1997 or 1998 experiments was determined (Fig. 8). The allometric coefficient K (the slope of the relationship between log$_e$ g dry weight root and log$_e$ g dry weight shoot) did not significantly differ among any of the treatments in the 1996 ($F = 0{\cdot}69$, $P > F = 0{\cdot}5627$) and 1998 ($F = 1{\cdot}02$, $P > F = 0{\cdot}3860$) experiments. There were significant differences among the slopes of the four treatments in 1997 ($F = 3{\cdot}93$, $P > F = 0{\cdot}0116$). In 1997, the O_3-exposed constant N treatment had a K coefficient significantly lower than the charcoal-filtered, constant N control, indicating an increased partitioning to the shoot. The other treatments did not differ in their *K*-values from the control.

DISCUSSION

This study investigated the interaction between O_3 and N availability. Two hypotheses were tested: (1) N remobilized

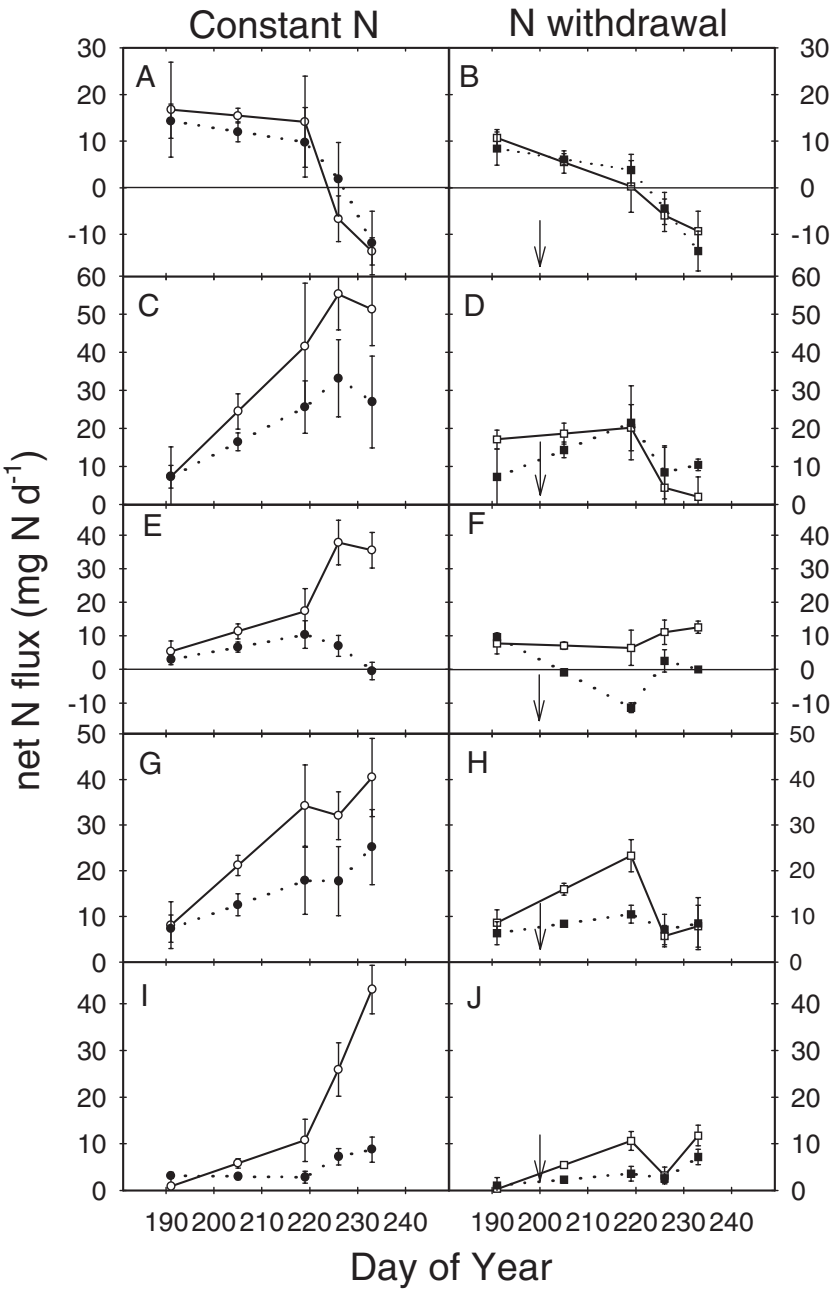

Figure 6. Nitrogen flux into upper, expanding leaves (A, B), middle leaves (C, D), lower leaves (E, F), stem and petioles (G, H), and roots (I, J) of hybrid poplar grown in charcoal-filtered (CF) air with (filled symbols, dotted lines) or without (open symbols, solid lines) supplementary O_3 in the 1997 experiment. Treatments and symbols are as in Fig. 4. Values represent the mean of four replicates ± SE.

during O_3-induced accelerated senescence of older leaves is incorporated by young leaves, and (2) O_3 stress will exacerbate N deficiency by decreasing root biomass allocation. The first hypothesis was found to be conditionally true; the second hypothesis was not supported.

Effect of O_3 and decreased N availability upon N allocation

Plants exposed to declining N availability along with O_3 stress had greater O_3-induced accelerated leaf senescence (Bielenberg *et al*. 2001). We hypothesized that remobilization of N from leaves as a result of O_3-induced accelerated senescence would supply a greater proportion of the N used by new leaves. As labelling a specific N pool *in planta* may result in a disturbance of the pool being labelled, the activity of the remobilized N by examination of N newly acquired by the plant was assessed. Specific allocation (mg new N per mg of total N) of newly acquired N to upper leaves was strongly reduced by N withdrawal (Fig. 1). However, specific allocation of new N to upper leaves was not further reduced in the N withdrawal treatment by O_3 exposure (Fig. 1). This is despite the N flux data from the 1996 and 1997 experiments indicating a large negative flux of N was occurring in the lower leaves of the O_3-exposed N withdrawal treatment, presumably making this pool of N

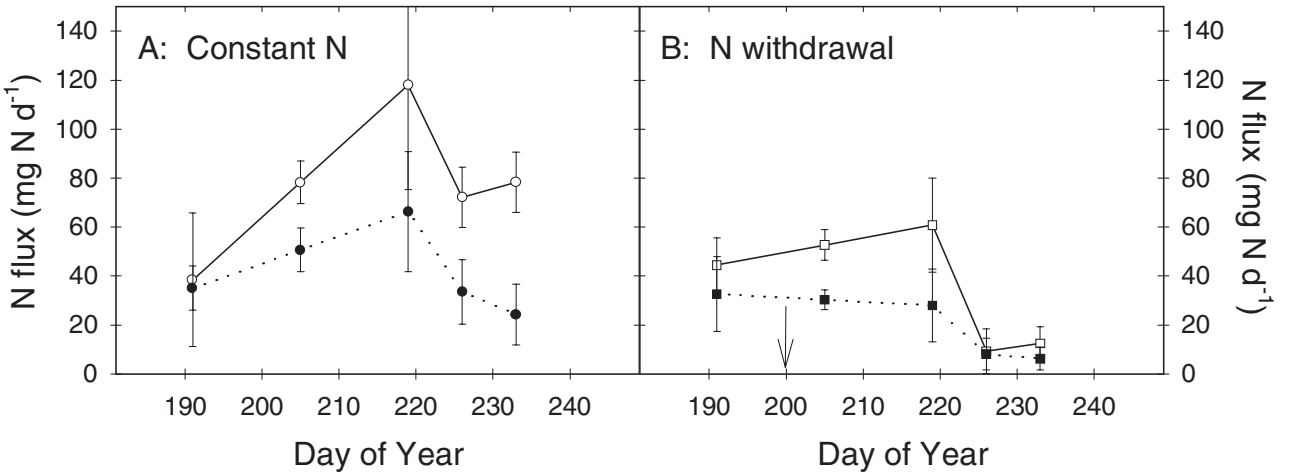

Figure 7. Whole plant N uptake (mg N d^{-1}) during the course of the 1997 experiment. Hybrid poplars were grown in charcoal-filtered (CF) air with (filled symbols, dotted lines) or without (open symbols, solid lines) supplementary O_3. Plants were supplied with 3·57 mM N daily for the entire experiment (A) or were supplied with 3·57 mM N daily until approx. 4 weeks of O_3 exposure at which time they were reduced to 0·71 mM N daily (B). Values represent the mean of four replicates ± SE.

available for use in new leaf growth (Fig. 6F). That specific allocation of new N is unchanged in the upper leaves when O_3-induced accelerated senescence is occurring does not support the original hypothesis.

A partial explanation for the lack of an O_3 effect on specific allocation of new N to upper leaves was found when the relative partitioning of new N within the plant was calculated. The proportion of newly acquired N allocated to upper leaves had increased in response to O_3 exposure in both N treatments (Fig. 2A). This shift in allocation may have offset increases in allocation of remobilized N to new growth. Increased relative partitioning of new N to the

Table 2. Results of statistical analyses for effects of air (O_3) and N treatment (N) and harvest date (D) on net N flux (mg N d^{-1}) into plant fractions in the 1996 and 1997 experiment. Plant fractions are divided as described in the Materials and methods

		Source of variance						
		N	O_3	N × O_3	D	N × D	O_3 × D	N × O_3 × D
1996	d.f.	1	1	1	3	3	3	3
Upper	*F*	1·78	0·10	2·20	3·50	1·32	0·75	0·88
	P > *F*	0·2189	0·7568	0.1766	**0·0310**	0·2920	0·5315	0·4656
Middle	*F*	8·46	0·61	0·00	1·87	1·32	0·50	0·24
	P > *F*	**0·0196**	0·4579	0·9871	0·1624	0·2895	0·6862	0·8659
Lower	*F*	1·76	3·71	0·06	0·50	0·78	0·51	2·16
	P > *F*	0·2209	0·0903	0·8150	0·6875	0·5161	0·6761	0·1196
Stem	*F*	4·24	0·24	1·03	1·52	1·59	0·58	0·42
	P > *F*	0·0734	0·6342	0·3402	0·2356	0·2178	0·6327	0·7427
Roots	*F*	5·89	0·00	0·24	2·14	0·70	0·68	1·12
	P > *F*	**0·0414**	0·9503	0·6342	0·1212	0·5592	0·5734	0·3606
1997	d.f.	1	1	1	3	3	3	3
Upper	*F*	4·58	0·04	0·00	17·74	1·12	0·33	0·58
	P > *F*	**0·0536**	0·8403	0·9489	**0·0001**	0·3556	0·8008	0·6308
Middle	*F*	35·76	4·35	7·46	0·77	4·24	0·02	0·63
	P > *F*	**0·0001**	0·0590	**0·0182**	0·5166	**0·0116**	0·9954	0·5999
Lower	*F*	49·88	81·22	5·12	5·91	1·65	4·83	5·87
	P > *F*	**0·0001**	**0·0001**	**0·0430**	**0·0022**	0.1955	**0·0003**	**0·0023**
Stem	*F*	34·54	14·17	3·50	1·38	3·11	0·45	0·60
	P > *F*	**0·0001**	**0·0027**	0·0859	0·2651	**0·0383**	0·7161	0·6219
Roots	*F*	48·11	82·08	30·71	22·36	13·47	7·75	9·17
	P > *F*	**0·0001**	**0·0001**	**0·0001**	**0·0001**	**0·0001**	**0·0004**	**0·0001**

Bold *P*-values are ≤ 0·05.

Table 3. Whole plant N uptake (mg N g^{-1} DW d^{-1}) calculated from accumulation of labelled N in plants on day 208 of the 1998 experiment. (mean ± SE, n = 16).

Treatments	mg N g^{-1} DW d^{-1} uptake
Constant N, charcoal-filtered air	0·91a ± 0·10
Constant N, O_3-added air	0·97a ± 0·09
N withdrawal, charcoal-filtered air	0·51b ± 0·06
N withdrawal, O_3-added air	0·47b ± 0·10

Treatments with different letters are significantly different at a P-level ≤ 0·05.

young leaves fits a pattern of plant response to O_3 wherein shoot growth is promoted over root growth. However, partitioning of new N to the roots was also favoured by O_3 ($P > F$ = 0·0850, Table 1), especially in the N withdrawal treatment (Fig. 2B). The increased partitioning of new N to both the upper, expanding leaves and the roots in response to O_3 exposure might indicate that in response to O_3 stress, plants allocate more N to regions of active growth as opposed to simply favouring the shoot. Additionally, shifts in N allocation could be a function of the senescence of older leaves that is occurring in the O_3 treatments. Jordi *et al*. (2000) described an analogous effect of leaf senescence upon allocation of labelled N from the root environment. When senescence of older leaves was prevented by auto-regulated production of cytokinins, N allocation to new and expanding leaves was reduced.

Nitrogen withdrawal and O_3 exposure had similar effects on relative partitioning of new N to roots but different effects on relative partitioning to the shoot (Fig. 2). These differential effects determined the balance between partitioning to root and shoots in response to N treatment and O_3 (Fig. 3). The ratio of relative partitioning of newly acquired N to upper leaves to the relative partitioning of new N to roots, corrected for whole plant biomass, showed that O_3 did not affect the balance of N allocation between roots and new leaves (Fig. 3). The fact that expressing the relationship on a whole plant dry weight basis reduced variability in the relationship indicated a probable underlying allometric relationship, but this study did not contain a large enough range of data points to address this question. The effects of ozone upon allocation appeared to be mediated through effects upon whole plant growth. Nitrogen

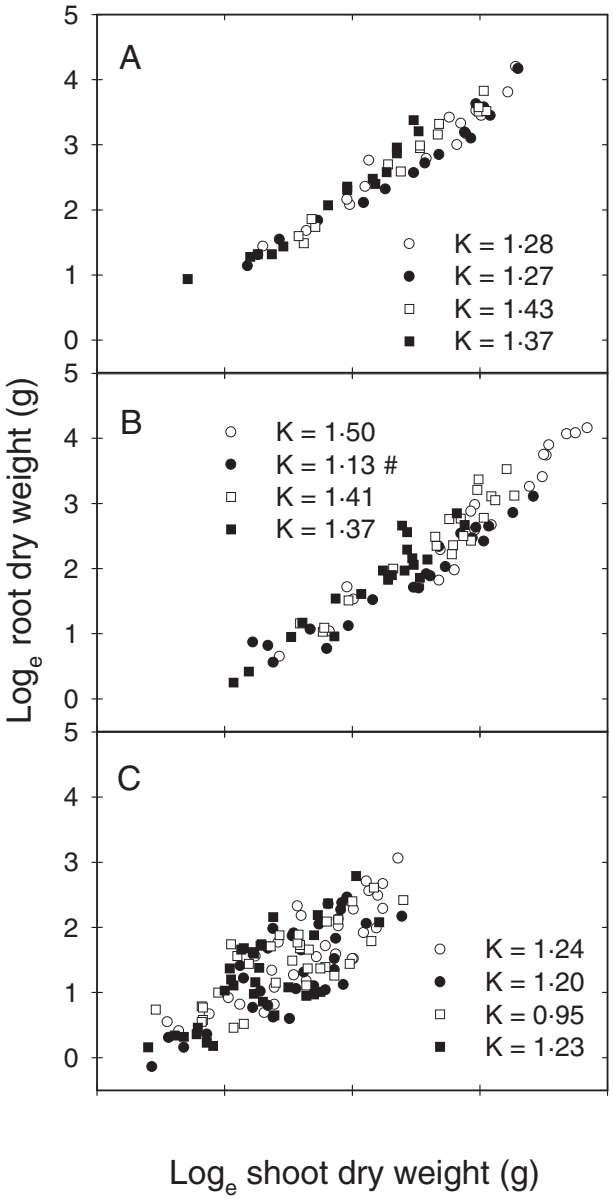

Figure 8. Allometric relationship between shoot (log$_e$ g DW) and root (log$_e$ g DW) growth in hybrid poplar in 1996 (A), 1997 (B), and 1998 (C). Values of the allometric coefficient K are shown for each treatment and year, a '#' symbol following a value indicates that it is significantly different ($P \leq 0{\cdot}05$) from the charcoal-filtered, constant N control (open circles). Symbols and treatments are as in Fig. 4.

Table 4. Results of statistical analyses for the effects of air (O_3) and N treatment (N) and harvest date (D) upon whole plant N uptake (mg N d^{-1}) in the 1996 and 1997 experiments. Uptake in 1996 and 1997 was determined by flux analysis of whole plant total N content at destructive harvests

Whole plant N uptake (mg N d^{-1}) Source of variance	d.f.	Whole plant 1996		Whole plant 1997	
		F	$P > F$	F	$P > F$
N	1	8·21	**0·0210**	47·18	**0·0001**
O_3	1	0·04	0·8409	22·36	**0·0005**
N × O_3	1	0·41	0·5404	7·58	**0·0175**
D	3	0·82	0·4977	0·342	0·7954
N × D	3	1·02	0·4025	2·69	0·0610
O_3 × D	3	0·56	0·6441	0·52	0·6722
N × O_3 × D	3	0·79	0·5088	1·34	0·2776

Bold P-values are ≤ 0·05.

withdrawal did affect the balance of N allocation between new leaves and roots, shifting relatively more new N to the roots. This result was consistent with the effect of N treatment upon the specific allocation of new N to the upper leaves (Fig. 1).

Specific allocation of new N to upper leaves was responsive to N availability but not O_3 exposure. The rate of whole plant growth and growth of new leaves is strongly controlled by N availability (Marschner 1995). Although O_3 induced the senescence of a large number of leaves and triggered the remobilization of the N resources in those leaves, it did not appear to affect the allocation and partitioning of that remobilized N except through effects upon whole plant size. In contrast, decreased N availability exerted specific changes on N allocation that favoured roots over shoots and did not appear to be mediated by plant size.

O_3 does not interact with N availability to reduce N uptake or alter allometric relationships

In 1998, where only one date was measured by direct label accumulation, only N withdrawal reduced N uptake on a dry weight basis. When net N uptake (mg N d^{-1}) was calculated at several dates throughout the experiment, it was reduced by decreased N availability in 1996, and O_3 and N interacted significantly in 1997. Differences seen in uptake (mg N d^{-1}) by the plants in 1997 (Fig. 7) are reflective of differences seen in whole plant biomass (Fig. 4). These results indicate that O_3 is not exerting an effect upon nutrient uptake via mechanisms such as reduced carbohydrate supply to the roots. Rather, N uptake is restricted by N availability and by plant size, upon which O_3 has an effect.

Coleman & McConnaughay (1995) have asserted that interpretations of biomass partitioning must be made with caution considering that shifts in root to shoot ratios may be the result of treatment-induced size differences. In general, the allometric relationship between the roots and the shoots was not affected by the treatments (Fig. 8). Although shifts in root-to-shoot ratio did occur during the course of the experiment (data not shown), the relationship between the $\log_e$ of shoot dry weight and root dry weight did not change during the experiment within any of the treatments in all 3 years of experiments (Fig. 8), indicating no shift in the balance of relative growth rates of the shoots versus the roots in any treatment. Differences seen in root-to-shoot ratio are therefore the effect of plant size. King, Pregitzer & Zak (1999) also detected no environmental effects upon root and shoot allometry in their study of four *Populus tremuloides* Michaux clones subjected to soil warming and two N availability regimes. Root and shoot allometry were determined to be under strong genetic control in their study. Possibly this is a general feature of the genus *Populus*.

Is whole plant growth a modulator of the interaction between N availability and O_3?

The analysis of N partitioning and flux, N uptake, and biomass partitioning presented above are supportive of the conclusion that N and O_3 interact in a fundamental manner to affect processes such as O_3-induced accelerated senescence and potentially compensatory responses of the plant. Bielenberg *et al.* (2001) previously reported that compensatory responses of young leaves to O_3 exposure only occurred when N available to the plant declined and the O_3-induced, accelerated senescence was most severe. Compensatory responses may be occurring as a result of a temporary developmental uncoupling between new leaf growth and the senescence of older leaves. Although the rate and degree of O_3-induced, accelerated senescence and abscission is tied, to some extent, to the growth or rate of growth of the plant, some O_3-induced senescence and abscission does occur regardless of plant growth rate (Bielenberg *et al.* 2001). Ozone therefore is inducing senescence and abscission independent of other plant or environmental factors. If the rate of growth (number of leaves or leaf mass) of new leaves is slowed by environmental factors that do not affect (or may even accelerate) the rate of senescence, the greater available pool of N could result in an increased concentration of N in the younger leaves. This increase may be a temporary phenomenon as the rates of leaf growth and senescence fluctuate.

CONCLUSION

The N remobilized as a result of O_3-induced, accelerated senescence appeared to enter a general pool of N cycling within the plant. This internally available N was then partitioned among growing regions according to size-dependent factors and N availability. The same can be concluded for the effects of O_3 and N availability upon plant N uptake. The potentially antagonistic effects of O_3 and N availability upon root and shoot biomass partitioning were not important in this system considering the insensitivity of the allometric relationship to the treatments.

ACKNOWLEDGMENTS

D.G.B. thanks Judith P. Sinn and Christian Vinten-Johansen for technical expertise and assistance in plant culture. This research was supported by NSF Grant IBN-961030 and NSF Training Grant BIR-9413204. This research was also supported in part by the Pennsylvania Agricultural Experiment Station and the Environmental Resources Research Institute, Contribution no. 2081, Department of Plant Pathology, The Pennsylvania State University.

REFERENCES

Bielenberg D.G., Lynch J.P. & Pell E.P. (2001) A decline in nitrogen availability affects plant responses to ozone. *New Phytologist* **151**, 413–425.

Brendley B.W. & Pell E.J. (1998) Ozone-induced changes in biosynthesis of Rubisco and associated compensation to stress in foliage of hybrid poplar. *Tree Physiology* **18**, 81–90.

Brouwer R. (1962) Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Sciences* **10**, 361–376.

Coleman J.S. & McConnaughay K.D.M. (1995) A non-functional interpretation of a classical optimal-partitioning example. *Functional Ecology* **9**, 951–954.

Cooley D.R. & Manning W.J. (1987) The impact of ozone on assimilate partitioning in plants: a review. *Environmental Pollution* **47**, 95–113.

Edwards N.T. (1991) Root and soil respiration responses to ozone in *Pinus taeda* L. seedlings. *New Phytologist* **118**, 315–321.

Erickson R.O. (1976) Modeling of plant growth. *Annual Review of Plant Physiology* **27**, 407–434.

Greitner C.S., Pell E.J. & Winner W.E. (1994) Analysis of aspen foliage exposed to multiple stresses: ozone, nitrogen deficiency and drought. *New Phytologist* **127**, 579–589.

Gutschick V.P. & Kay L.E. (1995) Nutrient-limited growth rates: quantitative benefits of stress responses and some aspects of regulation. *Journal of Experimental Botany* **46**, 995–1009.

Haynes R.J. (1986) Uptake and assimilation of mineral nitrogen by plants. In *Mineral Nitrogen in the Plant-Soil System* (ed. R. J. Haynes), pp. 303–378. Academic Press Inc., Orlando, FL, USA.

Jordi W., Schapendonk A., Davelaar E., Stoopen G.M., Pot C.S., De Visser R., Van Rhijn J.A., Gan S. & Amasino R.M. (2000) Increased cytokinin levels in transgenic P-SAG12-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. *Plant, Cell and Environment* **23**, 279–289.

Kickert R.N. & Krupa S.V. (1990) Forest responses to tropospheric ozone and global climate change – an analysis. *Environmental Pollution* **68**, 29–65.

King J.S., Pregitzer K.S. & Zak D.R. (1999) Clonal variation in above- and below-ground growth responses of *Populus tremuloides* Michaux: influence of soil warming and nutrient availability. *Plant and Soil* **217**, 119–130.

Krupa S.V. (1997) *Air Pollution, People, and Plants: an Introduction*. APS Press, St. Paul, MN, USA.

Lynch J. & White J.W. (1992) Shoot nitrogen dynamics in tropical common bean. *Crop Science* **32**, 392–397.

Manderscheid R., Jager H.J. & Kress L.W. (1992) Effects of ozone on foliar nitrogen metabolism of *Pinus taeda* L. and implications for carbohydrate metabolism. *New Phytologist* **121**, 623–633.

Marschner H. (1995) *Mineral Nutrition of Higher Plants.* Academic Press, Inc., San Diego, CA, USA.

Marschner H., Kirkby E.A. & Cakmak I. (1996) Effect of mineral nutrition status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *Journal of Experimental Botany* **47**, 1255–1263.

Noodén L.D. & Leopold A.C. (eds) (1988) *Senescence and Aging in Plants.* Academic Press, Inc., San Diego, CA, USA.

Nouchi I., Ito O., Harazono Y. & Kobayashi K. (1991) Effects of chronic ozone exposure on growth, root respiration and nutrient uptake of rice plants. *Environmental Pollution* **74**, 149–164.

Nouchi I., Ito O., Harazono Y. & Kouchi H. (1995) Acceleration of ^{13}C-labelled photosynthate partitioning from leaves to panicles in rice plants exposed to chronic ozone at the reproductive stage. *Environmental Pollution* **88**, 253–260.

Ojanperä K., Sutinen S., Pleijel H. & Selldén G. (1992) Exposure of spring wheat, *Triticum aestivum* L., cv. Drabant, to different concentrations of ozone in open-top chambers: effects on the ultrastructure of flag leaf cells. *New Phytologist* **120**, 39–48.

Pääkkönen E. & Holopainen T. (1995) Influence of nitrogen supply on the response of clones of birch (*Betula pendula* Roth.) to ozone. *New Phytologist* **129**, 595–603.

Pääkkönen E., Holopainen T. & Kärenlampi L. (1995) Ageing-related anatomical and ultrastructural changes in leaves of birch (*Betula Pendula* Roth.) clones as affected by low ozone exposure. *Annals of Botany* **75**, 285–294.

Pausch R.C., Mulchi C.L., Lee E.H., Forseth I.N. & Slaughter L.H. (1996a) Use of ^{13}C and ^{15}N isotopes to investigate O_3 effects on C and N metabolism in soybeans. I. C fixation and translocation. *Agriculture, Ecosystems and Environment* **59**, 69–80.

Pausch R.C., Mulchi C.L., Lee E.H. & Meisinger J.J. (1996b) Use of ^{13}C and ^{15}N isotopes to investigate O_3 effects on C and N metabolism in soybeans. II. Nitrogen uptake, fixation, and partitioning. *Agriculture, Ecosystems and Environment* **60**, 61–69.

Pell E.J. & Dann M.S. (1991) Multiple stress-induced foliar senescence and implications for whole-plant longevity. In: *Response of Plants to Multiple Stresses* (eds H. A. Mooney, W. E. Winner & E. J. Pell), pp. 189–204. Academic Press, Inc., San Diego, CA, USA.

Pell E.J., Sinn J.P., Eckardt N., Vinten-Johansen C., Winner W.E. & Mooney H.A. (1993) Response of radish to multiple stresses. II. Influence of season and genotype on plant response to ozone and soil moisture deficit. *New Phytologist* **123**, 153–163.

Pell E.J., Sinn J.P. & Vinten-Johansen C. (1995) Nitrogen supply as a limiting factor determining the sensitivity of *Populus tremuloides* Michx. to ozone stress. *New Phytologist* **130**, 437–446.

Pell E.J., Temple P.J., Friend A.L., Mooney H.A. & Winner W.E. (1994) Compensation as a plant response to ozone and associated stresses: an analysis of ROPIS experiments. *Journal of Environmental Quality* **23**, 429–436.

Reich P.B. & Lassoie J.P. (1985) Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. *Environmental Pollution* **39**, 39–51.

Samuelson L.J. & Kelly J.M. (1996) Carbon partitioning and allocation in northern red oak seedlings and mature trees in response to ozone. *Tree Physiology* **16**, 853–858.

Sandelius A.S., Näslund K., Carlsson A.S., Pleijel H. & Selldén G. (1995) Exposure of spring wheat (*Triticum aestivum*) to ozone in open-top chambers. Effects on acyl lipid composition and chlorophyll content of flag leaves. *New Phytologist* **131**, 231–239.

Temple P.J. & Riechers G.H. (1995) Nitrogen allocation in ponderosa pine seedlings exposed to interacting ozone and drought stresses. *New Phytologist* **130**, 97–104.

van der Werf A. & Nagel O.W. (1996) Carbon allocation to shoots and roots in relation to nitrogen supply is mediated by cytokinins and sucrose: opinion. *Plant and Soil* **185**, 21–32.

Received 30 May 2001; received in revised form 8 November 2001; accepted for publication 8 November 2001