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Nutrition and the ozone sensitivity of birch (*Betula pendula*)

II. Carbon balance, water-use efficiency and nutritional status of the whole plant

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Abstract Cuttings of a single birch clone (*Betula pendula*) were grown in field fumigation chambers throughout the growing season in either filtered air (control) or 90/40 nl O_3 l^{-1} (day/night), both regimes being split into high and low nutrient supply. High nutrition was neither advantageous for maintaining the photosynthetic capacity and life span of the leaves (see Maurer et al. 1997) nor for limiting the productive loss of the whole plant under O_3 stress relative to low-fertilized (LF) plants. However, nutrition determined, through carbon allocation and leaf turn-over, the way plants coped with O_3 impact. High leaf turn-over under O_3 stress related the carbon gain of high-fertilized (HF) plants to the photosynthesis of newly formed, intact leaves, although the foliage area remained reduced (shedding of O_3-injured leaves, inhibited branching). In contrast, the low leaf turn-over of LF plants reflected the maintenance of the O_3-injured leaves, causing high respiratory costs in the whole-plant carbon balance and a root/shoot biomass ratio as low as in the HF plants. Within the root system, the carbon allocation was determined by nutrition rather than ozone, whereas the water-use efficiency of the whole-plant carbon increment was lowered by ozone in both nutrient regimes. The relationship between biomass production and nutrient levels in the whole plant was hardly affected by ozone, with only the range of interaction being narrowed. Conditions requiring the maintenance of foliage rather than favoring the replacement of O_3-injured leaves may render trees more susceptible to shifts in the carbon allocation.

Key words *Betula pendula* · Ozone · Nutrition · Carbon balance · Water-use efficiency

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Introduction

The role of nutrition in the ozone (O_3) sensitivity of woody plants is still under debate (Matyssek et al. 1995a). Stimulation of the metabolic activity by high nutrition may raise the O_3 tolerance (Karnosky and Witter 1992; Pääkkönen and Holopainen 1995; Pell et al. 1995). Low nutrition may render plants rather insensitive to O_3 impact (Greitner and Winner 1989; Weinstein et al. 1991). In part I of this series (Maurer et al. 1997), we tested the hypothesis that high nutrition lowers the O_3 tolerance of the leaves of a birch clone (*Betula pendula*) which was known to be susceptible to O_3 stress (Matyssek et al. 1991, 1992; Günthardt-Goerg et al. 1993).

As leaves in low-fertilized birch plants (LF) withstood the impact of ozone longer than at high nutrition (HF), the hypothesis was verified. Given the similarity in stomatal conductance, O_3 uptake was comparable under both nutrient supplies even though stomatal density was raised by both ozone and low nutrition (Frey et al. 1996; Maurer et al. 1997), suggesting that the compensation of O_3 stress (through detoxification and repair processes) was more efficient in the LF plants. Levels of reduced ascorbate (A. Polle, personal communication) and $\delta^{13}C$ were higher in the leaves of LF plants under O_3 exposure, the latter effect being caused by the stimulated PEPC (phosphoenol pyruvate carboxylase) activity rather than by stomatal limitation on CO_2 uptake (Saurer et al. 1995; cf. Farquhar et al. 1989), indicating a high metabolic demand for substrate and energy (Wiskich and Dry 1985). Nevertheless, these leaves only delayed the incipient injury to leaf gas exchange. Later in the season they developed decreasing photosynthesis and water-use efficiency (WUE), and raised respiratory costs, similar to the corresponding HF treatment (Maurer et al. 1997). The observation that O_3-injured leaves are maintained longer under low rather than high nutrient supply may pose a distinct constraint on the whole-plant carbon allocation (Mooney and Winner 1991). The higher leaf formation rate in the HF plants, which was associated with a shorter life span of the O_3-exposed leaves, resulted

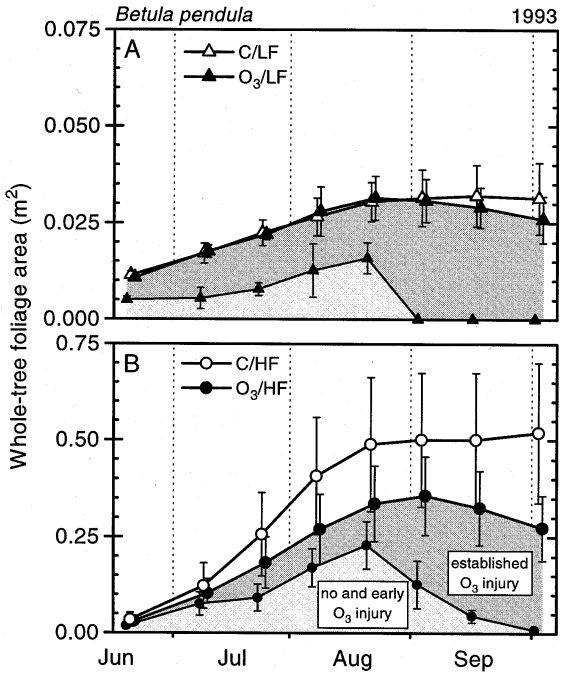

Fig. 1 Seasonal course of the whole-tree foliage area during 20 June through 6 October 1993. The whole-tree foliage area of the O_3-exposed trees (*closed, big symbols*) is divided into the proportion of intact leaves, including leaves with early stages of macroscopic injury (class {0|1}, according to Günthardt-Goerg et al. 1993; given as light-grey areas), and into the proportion of leaves with established O_3 injury (class {2|3}, given as dark-grey areas). *Open symbols* represent the 'controls' in O_3-free air. Mean and standard deviation were calculated from 5 trees per treatment (except for C/LF with 3 trees). Note that for graphical reasons the scales of **A** and **B** differ by a factor of 10. Treatment abbreviations as given in Table 1

in a raised leaf turn-over. The latter perhaps counteracted the productive limitation by ozone (cf. Tjoelker and Luxmoore 1991) so that at the whole-plant level, high nutrition may be beneficial under O_3 stress. Thus, the above hypothesis that high nutrition lowers O_3 tolerance needs to be questioned and examined again, but in part II of this series, in terms of the carbon balance, water-use efficiency and nutritional status of the whole plant.

Materials and methods

Plants and treatments

Cuttings of one birch clone (*Betula pendula* Roth; see Matyssek et al. 1991, 1992, 1995b; Günthardt-Goerg et al. 1993) were grown during the growing seasons (April through October) of 1992 and 1993 in the Birmensdorf field fumigation chambers (cf. Landolt et al. 1989; Maurer 1995; Saurer et al. 1995). In each year, a new set of 160 cuttings was used. Water was provided in a non-limiting supply through either a 0.05% or 0.005% fertilizer solution with macro- and micronutrients (80 plants/treatment; Hauert, Nährsalz Typ A, Anzucht). For further details on plant cultivation see Saurer et al. (1995) and Maurer et al. (1997). Each nutrient treatment was split into two O_3 exposures (i.e., 40 plants per ozone/nutrient regime) with either charcoal-filtered air (< 3 nl O_3 l^{-1}, control, regarded as 'O_3-free' in the following) or charcoal-filtered air enriched with ozone (40 nl O_3 l^{-1} from 2100 to 0700 hours, and 90 nl O_3 l^{-1} from 0700 to 2100 hours; generation and monitoring of ozone as given in Maurer et al. 1997). The treatment abbreviations used in the following are 'LF' for low-fertilized and 'HF' for high-fertilized plants, and 'C' for 'O_3-free and 'O_3' for ozonated air.

Biomass analysis

In each year, trees were randomly chosen and harvested on August 3 (5 trees/treatment; period of intensive shoot growth in HF plants), and on October 3 (10 trees/treatment; after completion of shoot growth but before onset of autumnal discoloration). The shoot biomass was analyzed by stem and branches, their corresponding foliage and by the stem section of the initial cutting. Additionally, the proportion of macroscopic O_3 injury in the foliage was determined according to the injury classes of Günthardt-Goerg et al. (1993): no/early O_3 injury = classes 0, 0.5 and 1 (abbreviated as {0|1} in the following text); established O_3 injury = classes 2 and 3 ({2|3}). The root biomass was analyzed by three diameter classes: fine (< 1 mm), medium-sized (1–2 mm) and coarse roots (> 2 mm), aliquots (root sections suspended in water) were taken for length measurement. Root length and foliage area were determined with the Delta-T, MK2 (UK) area/length meter. Biomass was dried at 65 °C to constant weight for 3 days.

After ranking the trees of the October harvest by whole-plant biomass, Ba, Ca, Fe, K, Mg, Mn, P and Zn were measured in all plant organs and biomass fractions of every second tree in each treatment with ICP-AES (Optima 3000, Perkin-Elmer), and carbon and nitrogen with a C/N analyzer (NA1500 analyzer, Carlo Erba). Whole-tree concentrations of these elements were calculated from the whole-tree biomass and the whole-tree element content yielded from the biomass fractions.

Table 1 Analysis of the stem and branch axes as measured at the beginning of October, 1993. Mean and standard deviation were calculated from 10 trees per treatment. [*C* = 'O_3-free' filtered air (control); *O3* = exposure to 90/40 nl O_3 l^{-1} (day/night); *LF* = low-fertilized, *HF* = high-fertilized plants.] The symbols by the values indicate significant differences between the treatments as specified: * C/LF ↔ C/HF or O_3/LF ↔ O_3/HF; ■ C/LF ↔ O_3/LF or C/HF ↔ O_3/HF (one symbol: $P < 0.05$; two symbols: $P < 0.01$; three symbols: $P < 0.001$)

	Treatments			
	C/LF	O_3/LF	C/HF	O_3/HF
A Number of branches	1.1± 1.7	0.3± 0.7	10.7± 4.7***	6.9± 2.3■/***
B Mean branch length (cm)	3.9± 0.6	5.1± 2.7	59.9±11.2**	50.9± 6.1*
C Mean stem length (cm)	39.3±10.9	46.9±12.3	184.1±19.2***	170.9±15.0***
D Number of leaves developed on the stem	14.6± 2.4	15.8± 3.0	35.9± 2.8***	35.7± 3.3***

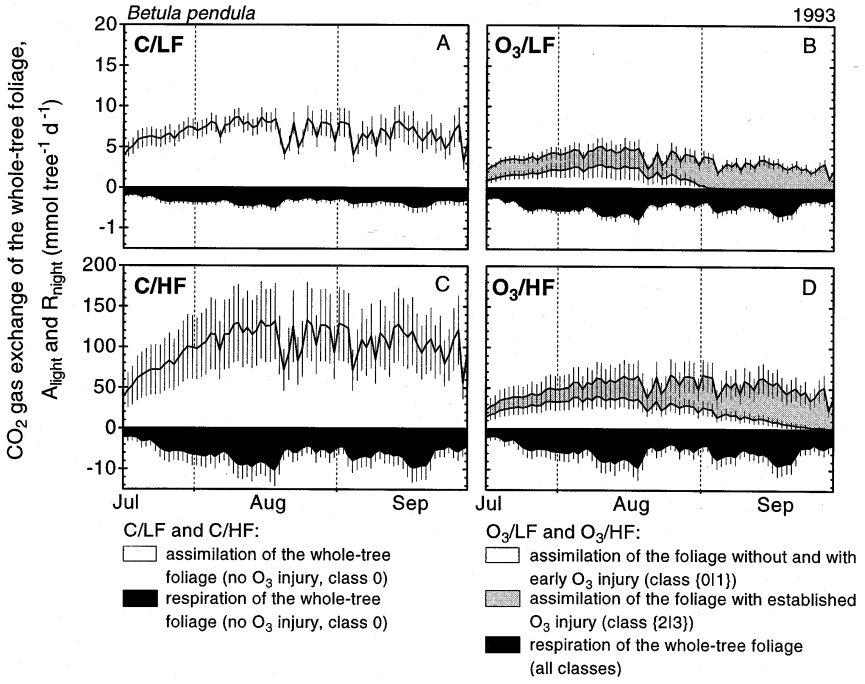

Fig. 2 Net CO_2 gain of the whole-tree foliage during the daily light hours, A_{light}, and dark respiration of the whole-tree foliage during the daily night hours, R_{night}, as calculated for the time period from 20 July through 3 October 1993. Note that, for graphical reasons, the scales in **A** and **B** differ by a factor of 10 from those in **C** and **D**. In each graph, the scale of the dark respiration has been enlarged by a factor of 5 relative to the corresponding A_{light}. A_{light} of the O_3-exposed trees is subdivided into foliage without and with incipient O_3 injury (class {0|1}) and into foliage with established O_3 injury (class {2|3}, see Fig. 1). Mean and standard deviation were calculated from 5 trees per treatment (except for C/LF with 3 trees). Treatment abbreviations as given in Table 1

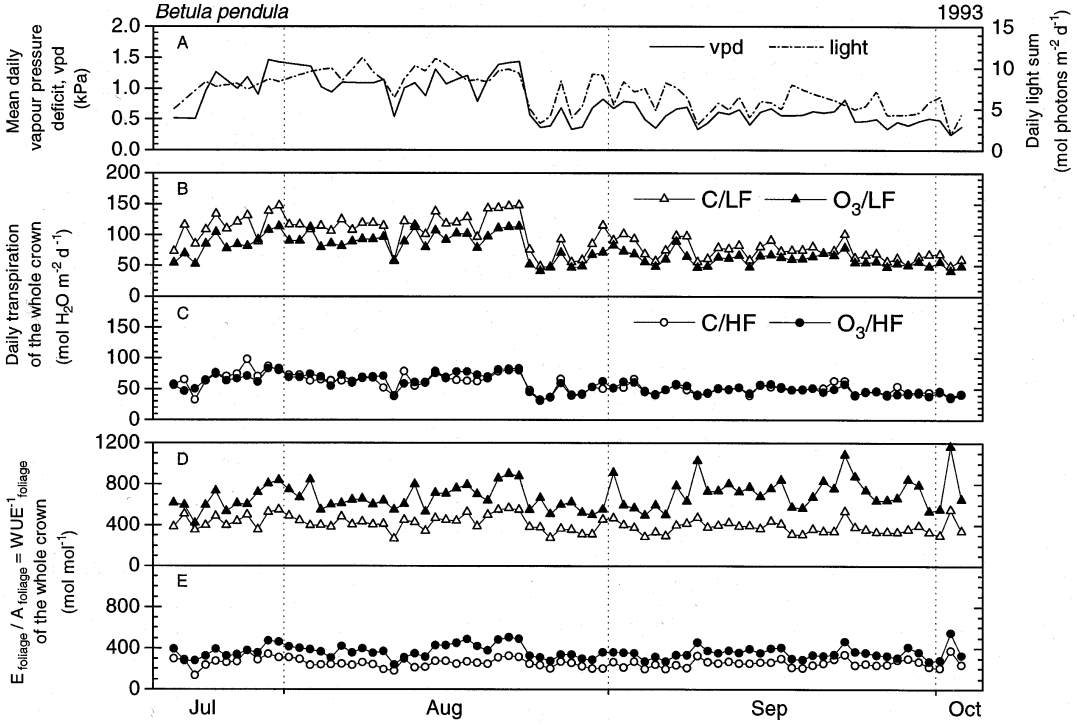

Fig. 3 Seasonal course of **A** daily irradiance (*PPFD*) and mean vapour pressure deficit (*vpd*) **B, C** daily transpiration of the whole crown, and **D, E** daily transpiration / net CO_2 gain of the whole crown, $E_{foliage}/A_{foliage}$ (i.e. $WUE^{-1}_{foliage}$) from 20 July through 3 October 1993. Each day, another tree per treatment was measured for 24 h (each datapoint represents one tree; see Materials and methods). Treatment abbreviations as given in Table 1

Transpiration and carbon gain of the whole plant

From July to October 1993, whole-plant transpiration and carbon gain were determined on a daily basis, using a total of five trees in each treatment. Each day, another tree was placed into a container which sealed the pot air-tight and provided ample water and air to the roots. The transpirational weight loss through the above-ground tree parts of this set-up was determined after 24 h (Mettler balance, PM30-K, CH); in the same way, four trees (one tree/treatment) were operated in parallel each day. The daily transpiration was related to the whole-plant foliage area and, on this area basis, calculated for the other four trees selected in each treatment. The foliage area at each day was estimated from non-destructive measurements performed at 2-week intervals using a portable leaf area meter (Licor, LI-3000A, USA), taking into account the proportion of the injured foliage area (according to the classes of leaf injury defined above).

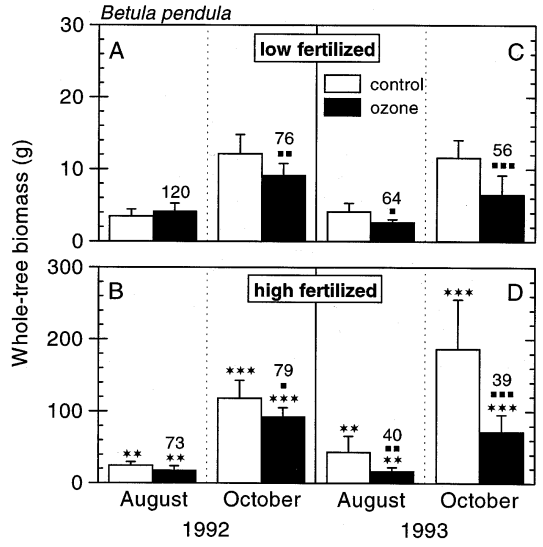

Fig. 4 Whole-tree biomass as measured at the beginning of August and at the beginning of October in 1992 (left) and 1993 (right). Mean and standard deviation (only one side shown) were calculated from 5 trees per treatment in August, and from 10 trees per treatment in October. The numbers above the columns of the O_3-exposed trees show the biomass in proportion to the corresponding control trees under the same nutrient regime. Symbols of significance levels as given in Table 1. Note that for graphical reasons the scales of **A** and **B** differ by a factor of 10

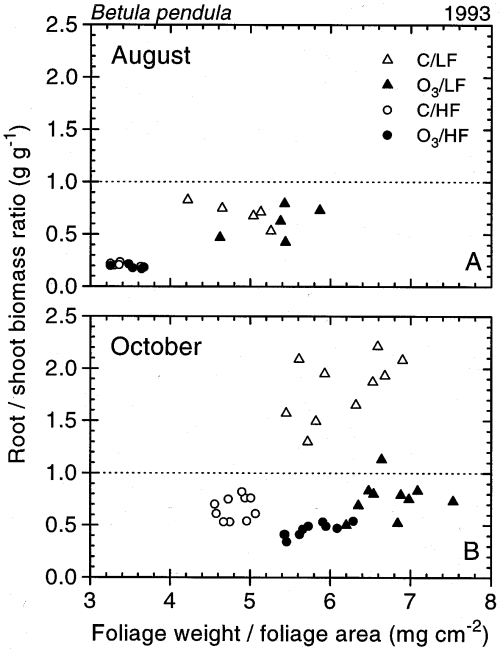

Fig. 5 Relationship between root/shoot biomass ratio (section of the initially planted cutting not included) and foliage weight / foliage area of the whole tree as measured at the beginning of August and October in 1993. Treatment abbreviations as given in Table 1

Table 2 Element concentrations in the whole-tree foliage and C/N ratio of the whole-tree as measured at the beginning of October 1993. Mean and standard deviation were calculated from 5 trees per treatment. Symbols of significance levels and treatment abbreviations as given in Table 1. For comparison, the values in the round brackets give the concentration ranges reported for birch leaves by Bergmann (1992)

	Treatments				
A	Macronutrients	C/LF	O_3/LF	C/HF	O_3/HF
N	[mg g^{-1}] (25–40)	18.9 ± 2.7	15.3 ± 1.8	33.0 ± 1.2**	25.7 ± 2.0■■/**
P	[mg g^{-1}] (1.5–3.0)	1.6 ± 0.3	1.7 ± 0.3	9.2 ± 1.2**	7.3 ± 0.6■/**
K	[mg g^{-1}](10–15)	9.8 ± 1.0	17.9 ± 2.4■■	27.1 ± 3.7**	27.6 ± 1.5**
Ca	[mg g^{-1}] (3–15)	16.3 ± 0.8	13.6 ± 0.7■■	7.7 ± 0.5**	6.9 ± 0.1■■/**
Mg	[mg g^{-1}] (1.5–3.0)	4.9 ± 0.5	4.2 ± 0.7	2.9 ± 0.2**	3.1 ± 0.2**
B	Micronutrients				
Mn	[µg g^{-1}] (30–100)	209 ± 83	169 ± 83	374 ± 77**	362 ± 40**
Fe	[µg g^{-1}] (–)	56 ± 10	59 ± 12	229 ± 31**	226 ± 88**
Zn	[µg g^{-1}] (15–50)	191 ± 53	124 ± 41	191 ± 45	175 ± 37
C	Barium				
Ba	[µg g^{-1}] (–)	110 ± 34	102 ± 25	18.2 ± 2.4**	16.7 ± 2.3**
D	C/N ratio of the whole tree				
	[mol C mol^{-1} N]	45.4 ± 6.3	33.0 ± 3.1■■	26.7 ± 1.5**	25.7 ± 1.2**

The carbon gain of the trees was calculated on the basis of their seasonal foliage area development, the diurnal courses in light and temperature inside the exposure chambers, the light dependence of the CO_2 uptake rate (relationships derived from the pooled datasets of the diurnal gas exchange of 5–8 leaves in each treatment (see Maurer 1995), and the temperature dependence of the dark respiration rate (see Maurer et al. 1997). In the O_3-exposed trees, the gas exchange behavior of 4-week-old leaves was assigned to the foliage area showing no or early stages of O_3 injury (i.e. class {0|1}, see above), and the gas exchange of 8-week-old leaves was assigned to the foliage area with established O_3 injury (class {2|3}). In the 'control' trees exposed to O_3-free air, half of the foliage area was assumed to behave in gas exchange like 4-week-old leaves, the other half like 8-week-old leaves. The gas exchange of leaves on branches was assumed to behave similar

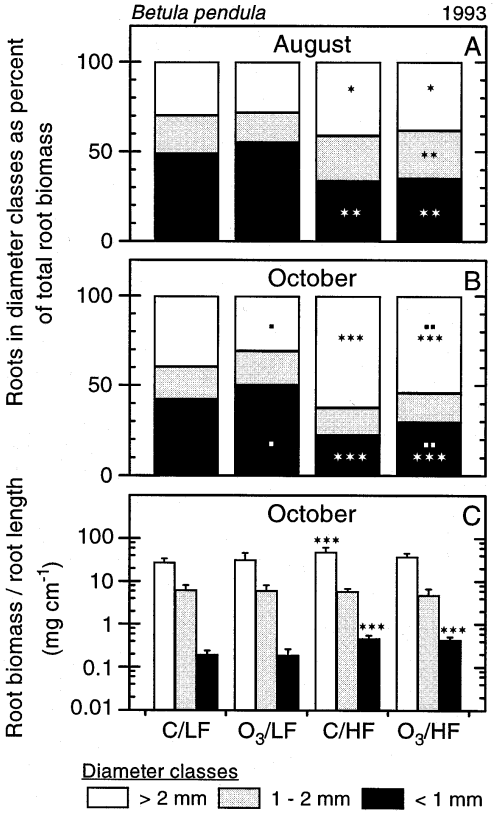

Fig. 6 A, B Proportion of coarse roots (> 2 mm in diameter), medium-sized roots (1–2 mm) and fine roots (< 1 mm) in the total root biomass as measured at the beginning of August and at the beginning of October in 1993. **C** Root biomass per root length of coarse, medium-sized and fine roots at the beginning of October in 1993. Mean and standard deviation (only one side shown) were calculated from 5 trees per treatment in August, and from 10 trees per treatment in October. Symbols of significance levels and treatment abbreviations as given in Table 1

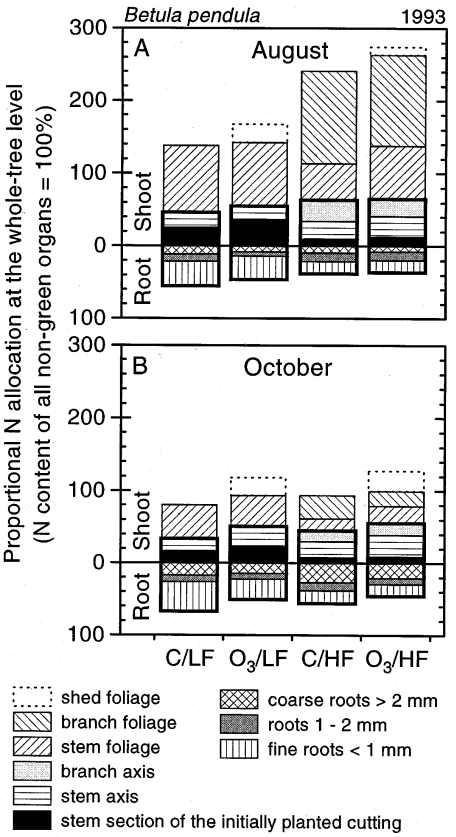

Fig. 7 Nitrogen (*N*) allocated to the different tree organs at the beginning of August and at the beginning of October in 1993. The N content of all non-green organs has been set to 100 % (indicated as the thick frame around the corresponding fractions of the columns), which is the basis for expressing the N proportion allocated to the different kinds of organs. Mean values were calculated from 5 trees per treatment. Treatment abbreviations as given in Table 1

Table 3 Whole-tree carbon balance and transpiration during the time period from 3 August through 3 October 1993. Mean and standard deviation were calculated from 5 trees per treatment (except for C/LF with 3 trees). Symbols of significance levels and treatment abbreviations as given in Table 1

	Treatments			
	C/LF	O_3/LF	C/HF	O_3/HF
Net CO_2 gain of the whole-tree foliage during the light hours, ΣA_{light} [mmol]	430.7 ± 75.1	220.9 ± 43.5■	6758.3 ± 2396.1**	3330.0 ± 994.4■/*
Contribution to ΣA_{light} by foliage with established O_3 injury (class {2|3}) [%]	–	70.5 ± 11.1	–	57.9 ± 3.2*
Carbon incorporated in biomass increment in relation to ΣA_{light} [%]	67.0 ± 9.8	48.0 ± 8.1	66.9 ± 6.5	73.5 ± 6.4*
Foliage respiration during the night hours in relation to ΣA_{light} [%]	5.3 ± 0	14.0 ± 0.8■	6.2 ± 0.0**	11.3 ± 0.1■■/*
Respiration of non-green biomass in relation to ΣA_{light} [%]	27.7 ± 9.7	38.0 ± 7.6	26.8 ± 6.5	15.1 ± 6.3■/*
Transpiration/carbon incorporated in biomass increment [mol H_2O mol^{-1} C]	569 ±93	1240 ±220■	354 ± 35**	438 ± 37■/*

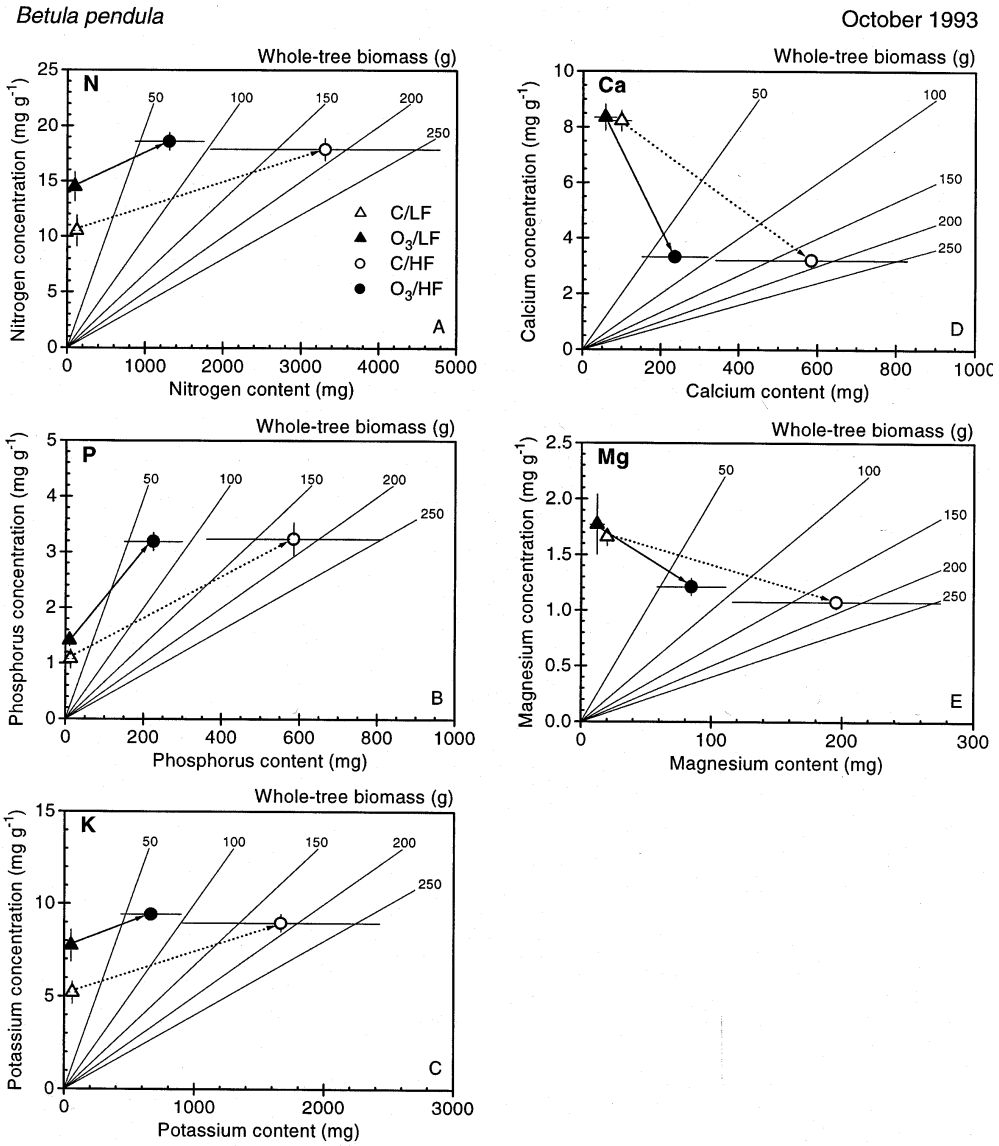

Fig. 8 Effect of nutrient supply on the whole-tree content and whole-tree concentration of N, P, K, Ca and Mg as well as on the whole-tree biomass production as measured at the beginning of October in 1993. Mean and standard deviation were calculated from 5 trees per treatment. The diagonals represent the whole-tree biomass. The vectors connect low and high-fertilized trees of each O_3 regime, the vector direction indicating the change in the nutritional status due to additional fertilization (concept by Timmer and Morrow 1983, applied for the indeterminate shoot growth of birch). Treatment abbreviations as given in Table 1

to that of the leaves on the stem. This was supported by measurements of 3 to 10 branch and stem leaves on different trees in each treatment during early September, using a portable CO_2/H_2O diffusion porometer (Walz, Germany) under ambient conditions (Maurer 1995).

Whole-tree carbon balance

From 3 August to 3 October, 1993, the net photosynthetic carbon gain during the light hours, dark respiration and transpiration of the foliage were calculated as the seasonal integrals of the corresponding daily determinations (see above). For the same time period, the whole-plant biomass increment of the trees harvested in October was calculated by subtracting their estimated dry weight on August 3 from their actually measured dry weight on October 3. The estimation of the dry weight on August 3 was based on the mean '*net-assimilation rate*' (i.e. the '*whole-tree biomass/whole-tree foliage area*' ratio) of the other trees harvested in each treatment on August 3. This latter ratio was multiplied by the foliage area on August 3 of the trees harvested in October (Maurer 1995). Biomass losses caused by premature leaf shedding were taken into account by adding the difference between the biomass of shed leaves (given by the number of shed leaves multiplied by the mean '*leaf weight/tree*' ratio) determined on August 3 and October 3 to the whole-plant biomass increment. The biomass increment multiplied by 0.48 g C $g^{-1}_{dry\text{-}weight}$ (i.e. the mean carbon concentration of the whole tree) provided the whole-tree carbon increment, which is compared with the seasonal transpiration and carbon gain (the difference between the amount of carbon in the whole-tree biomass increment and the whole-tree photosynthetic carbon gain is regarded as respiratory loss).

Statistics

The paired Kruskal-Wallis test was used to statistically examine treatment differences (StatGraphics Plus for Windows 1.0; Statistical Graphics). As the findings were similar in 1992 and 1993, data presentation is restricted mainly to the 1993 growing season.

Results

Seasonal dynamics in foliage development, whole-plant carbon gain and water loss

In the LF plants, the branch formation was low, and the whole-tree foliage area was only 10% of that in the HF plants (Fig. 1). However, the foliage area of LF plants was largely unaffected by ozone throughout the growing season (Fig. 1A). In contrast, the foliage area of HF plants remained low under O_3 exposure (Fig. 1B) as a consequence of inhibited branch formation and premature leaf loss (Table 1A; Maurer et al. 1997). Under both nutrient regimes, ozone affected neither the length growth nor the leaf formation on the stem (Table 1C,D) nor the mean branch length (Table 1B). By the end of August, leaves without O_3 symptoms and leaves displaying incipient injury dominated the foliage area of the O_3-exposed HF trees, and the extent of O_3 injury gradually spread over the rest of the growing season (Fig. 1B). In contrast, established injury governed the major part of the foliage area in the O_3-exposed LF plants and spread across all leaves by early September (Fig. 1A). Neither nutrient regime prevented the daily carbon gains of O_3-exposed trees from declining distinctly below the gains of the controls in O_3-free air (Fig. 2), but in the HF plants the contribution of the leaves without symptoms (including the leaves with incipient injury) to the carbon uptake under O_3 exposure was high. At the foliage level, ozone raised the respiratory carbon release at night only in the LF trees, with their high proportion of severely injured leaves. According to the stomatal behavior (Maurer et al. 1997), only the daily foliage transpiration rate of LF plants declined under O_3 impact and approached the level of the water loss in HF plants (Fig. 3B,C). Nevertheless, the daily water loss of the foliage, when related to the carbon gain, was enhanced by ozone, independent of nutrition, although this decline in water-use efficiency (WUE) was most pronounced in the LF plants (Fig. 3D,E).

Biomass production and whole-plant allocation of carbon and nutrients

The biomass accumulated by August or October was about 10 times larger in the HF than in the LF plants, closely reflecting the difference in the nutrient supply (Fig. 4). Under O_3 exposure, however, the proportional reduction in the biomass production (as related, at each nutrient regime, to the biomass production of the corresponding control in O_3-free air) was similar (in 1992) or even smaller (in 1993) by October at low rather than high nutrient supply. By August, no O_3-induced shift was detectable in the root/shoot biomass ratio (R/S) of either nutrient regime, although R/S and the foliage weight/area ratio was higher in LF plants (Fig. 5A). By October, R/S increased to > 1 in LF plants under O_3-free air, whereas ozone caused R/S to remain similar to HF plants (Fig. 5B). In HF plants, ozone only slightly limited R/S relative to the corresponding control, whereas foliage weight/area tended to increase under O_3 impact regardless of nutrition. Although ozone limited root growth, the proportions of coarse, medium-sized and fine roots in the entire root biomass were only marginally affected by ozone (Fig. 6 A,B). However, high nutrition favored the formation of coarse roots, and low nutrition the fine-root production. Accordingly, fine and coarse root biomass was increased on a root length basis by high nutrient supply, in the absence of clear effects of ozone (Fig. 6 C).

By October, low nutrient supply and ozonation lowered the nitrogen concentration of the whole foliage, while the HF plants reflected the concentration range reported by Bergmann (1992) for birch leaves (Table 2). The foliage concentrations of P, Mg, Mn and Fe were only marginally influenced by ozone in either nutrient regime, whereas in the LF plants ozonation caused elevated K concentration. Unlike the other elements, Ca and Ba concentrations were distinctly higher in the LF than in the HF trees, while O_3 effects remained vague. The C/N ratio of the whole tree was reduced by high nutrient supply, whereas O_3 exposure lowered this ratio only in the LF trees (Table 2). Independent of the O_3 exposure, in August, HF plants allocated nitrogen to the foliage about twice of the non-green biomass, whereas in LF trees nitrogen was partitioned between the green and non-green organs to similar extents (Fig. 7A). By October, the proportion of nitrogen allocated to the foliage declined and was similar in all treatments (Fig. 7B). However, considering nitrogen loss through leaf shedding, the O_3-exposed trees invested relatively more nitrogen into the foliage, apparently at the expense of the fine (in O_3/LF) or coarse-root fraction (in O_3/HF) as compared with the corresponding controls in O_3-free air. The whole-tree content and concentration of N, P, K, as well as the whole-tree biomass increased at high nutrient supply (Fig. 8). These relationships indicate '*deficiency*' of these three elements in the LF trees, when applying the concept of Timmer and Morrow (1983) to the indeterminate shoot growth of birch. The whole-tree contents of Ca and Mg were also increased by high nutrition, however, their decrease in concentration reflects a '*non-limiting*' Ca and Mg status in the LF trees (cf. Timmer and Morrow 1983). Ozone did not change the trends but reduced the ranges of interaction in these interrelationships between biomass production and nutrition.

Whole-tree carbon balance and water loss

The net carbon gain of the foliage achieved during the daylight hours in late summer was reduced in both nutrient regimes by about 50% by ozone (Table 3). In the O_3-exposed LF plants, 71% of their gain was contributed by leaves with established injury, whereas the carbon fixation depended less on O_3-injured leaves in the HF plants (these trends establishing even more clearly, if the entire growing season was considered). O_3-exposed LF plants used proportionally more of their carbon gain for respiration and invested less into the biomass increment than did plants of the other treatments. Water loss, as based on the carbon allocated to the biomass increment, increased under O_3 stress, and this decline in the WUE of the biomass production was most pronounced in the O_3-exposed LF plants.

Discussion

Judging the O_3 tolerance of a plant depends on the plant process under consideration. The leaf life span of HF plants was shortened and decline was accelerated by ozone, but in LF plants leaf metabolism responded more sensitively to ozone, although in processes aiding maintenance (Saurer et al. 1995; Maurer et al. 1997; Landolt et al. 1997; A. Polle personal communication). At the whole-tree level, the proportional decline in the biomass production of HF plants caused by ozone was similar or even higher than at low nutrition (cf. Fig. 4), although LF plants were more sensitive in terms of their carbon allocation. Thus, judging by leaf life span and whole-plant production, the O_3 tolerance of the birch clone was not enhanced by high nutrition, which verifies the research hypothesis also at the whole-plant level but conflicts with findings by Pääkkönen and Holopainen (1995) for *Betula pendula* and Karnosky and Witter (1992) and Pell et al. (1995) for *Populus tremuloides*. Rather, low nutrition tended to lower O_3 sensitivity similar to *Pinus taeda* (Tjoelker and Luxmoore 1991) and *Salix nigra* (Greitner and Winner 1989).

Whole-plant carbon allocation and leaf turn-over were key processes relating nutrition to tree performance under O_3 impact. Low nutrition limited shoot growth and leaf formation, thus O_3-injured leaves could not be compensated for by new leaf growth. Instead, leaf shedding was delayed (Maurer et al. 1997). As a consequence, the foliage area was maintained throughout the growing season, and the photosynthesis of injured leaves determined the carbon gain. This achievement probably demanded for repair and detoxification, which requires additional assimilates in the shoot at the expense of the root and may lead to the reduced R/S by October (cf. Cooley and Manning 1987; Chappelka and Chevone 1992; Matyssek et al. 1995a). In fact, the leaf metabolism appeared to be stimulated in terms of elevated levels of antioxidants (A. Polle, personal communication), sugars (Landolt et al. 1997), PEPC activity and $\delta^{13}C$ (Saurer et al. 1995) and dark respiration. Apparently, the increasing R/S commonly associated with low nutrition was '*overwhelmed*' by O_3 stress. This perhaps enabled the 'set-point' between the plant-internal carbon and nutrient fluxes to be maintained and to limit productive loss by ozone (Mooney and Winner 1991). Such behaviour was not found by Greitner and Winner (1989) in low-fertilized *Salix nigra*. However, the severity of nutritional stress apparently determines the response to ozone, because R/S in *S. nigra* behaved similarly to our LF birches when nutrition was less restrictive. Also, the O_3 exposure of willow lasted for only 1 month, however, both ontogentic stage and O_3 dose govern R/S (Matyssek et al. 1992, 1993; cf. Körner 1996), as underlined in birch by the absence of an O_3 effect on R/S in August. Impeded assimilate export from the leaves due to O_3-induced mesophyll collapse and disorders in phloem loading and transport may have contributed to the lowered R/S found in the LF birch plants in October (Günthardt-Goerg et al. 1993; Landolt et al. 1997; cf. Spence et al. 1990). Given the lowered below-ground carbon allocation, however, the biomass partitioning and allometric differences between roots of different diameter was hardly influenced by ozone. The proportional decrease of the coarse roots in the entire root biomass in October possibly indicated low reserve storage as a consequence of the O_3 impact on the leaves.

High nutrition enhanced shoot growth and leaf formation. This allowed O_3-injured leaves to be replaced by new leaves and resembled compensations in *Populus tremuloides* (Brendley et al. 1994) or in the O_3-tolerant birch clone studied by Pääkkönen et al. (1993). The proportion of injured foliage area was, therefore, smaller in HF than in LF birch plants. However, the foliage area remained low in the O_3/HF plants, because ozone did not increase leaf formation beyond that of the control, as opposed to *Populus deltoides* × *trichocarpa* (Reich and Lassoie 1985) and *Populus deltoides* × *nigra* (Woodbury et al. 1994). Thus, the carbon gain was lowered in the O_3/HF plants (cf. Bassmann and Dickmann 1982, 1985; Woodbury et al. 1994) but relied on the photosynthesis of the non-injured leaves. Since both the metabolic demand for maintaining the injured leaves and their proportion in the total foliage were low, R/S was hardly affected by ozone in the HF plants.

Whole-tree concentrations of N, P, K, Ca and Mg were not changed by O_3 exposure in the HF but increased in the LF plants. This increase perhaps reflected the carbon balance which, relative to the O_3-exposed HF plants, displayed lower proportional biomass increment at raised respiratory costs in late summer. At the foliage level of the HF trees, however, some nutrient concentrations (N, P, Ca) were decreased by ozone, possibly because of changed leaf differentiation (Günthardt-Goerg et al. 1993) leading to increased foliage weight/area. In addition, nutrient retranslocation may have occurred *prior* to leaf shedding. In the foliage of LF plants, nutrient concentrations remained rather stable under O_3 stress, which appears to be consistent with the maintenance of the injured leaves. Contradicting nutrient responses to ozone may be explained on the basis of the nutritional status of the plants (Matyssek et al. 1995a). Regardless of ozonation, LF plants had increased levels of Ca and Ba, both of which can be indicative of high transpiration (Ziegler 1983; Jurat and Schaub 1988). High transpiration was indeed measured at the leaf (Maurer et al. 1997) and foliage level and may have supported the nutrient uptake, given the non-limiting water supply (Glatzel 1973). Thus, WUE of the carbon accumulation by the whole-plant biomass production was lower in LF than in HF trees, the latter trees resembling other woody plants in these terms (Matyssek and Schulze 1988), but WUE was further decreased by ozone, independent of nutrition. Applying the analytical concept of Timmer and Morrow (1983), the interrelationship between nutrition and production did not appear to be fundamentally changed by ozone, only the range of interaction became narrowed.

In conclusion, for the birch plants studied, high nutrition was of no advantage for withstanding O_3 stress, but nutrition determined the '*strategy*' of coping with the stress *via* driving the turnover of the leaves. The lower the leaf formation rate, as with low nutrition, the higher the need

for leaf maintenance and the greater the shift in whole-plant carbon allocation. High nutrition enabled high leaf turnover, which limited the productive decline by relating production to the photosynthesis of new leaf growth, as concluded by Tjoelker and Luxmoore (1991). Nevertheless, both '*strategies*' did limit productive loss to similar extents, with the LF plants tending to be even more efficient than the HF plants (cf. Weinstein et al. 1991). Conditions that require the maintenance of foliage rather than favor the replacement of O_3-injured leaves may render trees more susceptible to shifts in the whole-plant carbon allocation, to limitations in root growth and, eventually, to changes in competitiveness.

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