Ozone impairs autumnal resorption of nitrogen from birch (Betula pendula) leaves, causing an increase in whole-tree nitrogen loss through litter fall

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Summary  Saplings of one half-sib family of birch, Betula pendula Roth, were exposed to three ozone concentrations (non-filtered air (NF); non-filtered air + 10–20 nmol O3 mol–1 (NF+); non-filtered air + 40–60 nmol O3 mol–1 (NF++)) in open-top chambers during two growing seasons from 1997 to 1998. Shed leaves were collected regularly during both growing seasons and, in 1998, the dry mass (DM) and nitrogen (N) concentrations ([N]) of the shed leaves were measured to quantify the total amount of N lost through litter fall. Dry mass and [N] were also determined in mid-August for attached, mature and non-senescent leaves, in order to estimate autumnal leaf N resorption efficiency and proportional leaf DM decrease. Net photosynthetic capacity was measured during August and September 1998, in a population of leaves that emerged in mid-July. Photosynthesis declined with increasing leaf age in the NF++ treatment, whereas it remained high throughout the measurement period in the NF and NF+ treatments. In both years, leaves abscised prematurely in the NF++ treatment, whereas this effect was only significant in 1998 in the NF+ treatment. There was a strong linear relationship between proportional leaf shedding and daylight ozone exposure above a threshold of 40 nmol mol–1 (daylight AOT40) during the growing season. The resorption of N was significantly impaired by ozone, and the smaller autumnal decrease in leaf DM in elevated ozone concentrations suggested that the bulk resorption of leaf DM was also inhibited. Nitrogen resorption efficiencies were 81, 73 and 63% and leaf mass decreases were 45, 36 and 30% in the NF, NF+ and NF++ treatments, respectively. Compared with the NF treatment, total N loss through litter fall was increased by 16 and 122% in the NF+ and NF++ treatments, respectively. We conclude that ozone impaired N resorption from birch leaves before abscission, causing a substantial increase in whole-tree N loss through litter fall.

Keywords: abscission, nitrogen resorption efficiency, photosynthesis, senescence.

Introduction  
Autumnal leaf senescence is the final stage of leaf development and can be defined as “the series of events concerned with cellular disassembly in the leaf and the mobilization of materials released during this process” (Thomas and Stoddart 1980). Senescence events involve considerable de novo synthesis of proteolytic enzymes and other compounds. The transcriptional timetable for autumnal leaf senescence in Populus, involving a shift from photosynthetic competence to energy generation, was recently investigated (Andersson et al. 2004). The primary value of leaf senescence to plant fitness is the resorption and re-use of breakdown products (Aerts 1996). Autumnal nutrient resorption is a key component of the nutrient cycle in temperate hardwood forests, with trees typically resorbing half (Aerts 1996) or more (Duchesne et al. 2001, Côté et al. 2002, García-Plazaola et al. 2003) of the nitrogen (N) in the leaves before abscission. This resorbed N is an important source of N for growth in the following years (Aerts 1996). Internal cycling accounted for 37% of the N required for leaf growth of Betula pendula Roth seedlings (Millard et al. 1998). The proportion increases as trees grow larger, with about half of the N required for the total growth of B. pendula during a 45-year rotation estimated to come from internal cycling (Miller 1984). Remobilized N can be used for growth during periods when root uptake is low, thereby temporally decoupling growth and root uptake. Using 15N, Millard et al. (1998) showed that N derived from root uptake did not appear in the leaves of B. pendula until 12 days after bud burst. Until then, leaf growth relied exclusively on the remobilization of N from internal sources.

The impact of tropospheric ozone on vegetation has long been a major concern in North America as well as in Europe (Percy et al. 2003). At present, about a quarter of the global area occupied by forest is exposed to ozone concentrations exceeding 60 nmol mol–1 (Fowler et al. 1999), and the concentrations are expected to increase in the coming decades (Johnson et al. 2001). During the last decades, there have been many
studies on the effects of ozone on deciduous trees. In both chamber and open-air experiments, frequently observed effects include visible foliar symptoms, inhibition of photosynthesis and growth, and earlier abscission of leaves (Karnosky et al. 2003 and references therein). Although ozone-induced changes in leaf structure and biochemistry have been frequently studied (for birch, e.g., Günthardt-Goerg et al. 1993, 1997, Pääkkönen et al. 1995), little is known about how these changes affect nutrient resorption from leaves before abscission, and hence the total nutrient loss through litter fall. If ozone decreases leaf N resorption efficiency (NRE), a significant pool of N for new growth during the following years is lost and growth will become more dependent on current root uptake.

The aim of our study was to investigate the effects of ozone on senescence of B. pendula leaves, with the main focus on N economy. We investigated the hypotheses that elevated ozone causes (1) decreased photosynthetic capacity, (2) earlier leaf abscission, (3) decreased NRE and (4) increased total N loss through litter fall.

Materials and methods

The experiment was carried out from 1997–1998 in the Östad field station, located 50 km northeast of Göteborg, Sweden (57°54' N, 12°24' E; 62 m a.s.l.). Details of the field station as well as the plot design are described by Wallin et al. (2002). More detailed descriptions of the experimental design, plant cultivation, ozone fumigation and measurements of ozone and climate in the B. pendula ozone experiment can be found in Karlsson et al. (2003).

Plants and experimental design

The plant material came from seed orchard Ekebo2 and consisted of one commercially used half-sib family of Betula pendula, obtained from Clone S21K883060 (SkogForsk, Ekebo, Sweden). Seeds were sown in January 1997 and the seedlings were cultivated in greenhouses at SkogForsk, Ekebo, Sweden. The seedlings were planted in each plot, and half of the plants were harvested in early November after each growing season.

Measurements

Net photosynthetic capacity was measured with an LI-6200 portable photosynthesis system (Li-Cor, Lincoln, NE) with a QB6200 LED light source (Quantum Devices, Barneveld, WI). Each leaf was acclimatized to 1300 μmol photons m⁻² s⁻¹ for 5 min before measurement. The sampled leaf population consisted of mid-canopy leaves that emerged in mid-July 1998. The leaves were evenly distributed among the trees and tagged with colored threads. Gas exchange measurements were made weekly on one leaf from each of three trees per plot during August–September. The plots were sampled in a randomized order between 0900 and 1500 h. After each gas exchange measurement, the sampled leaf was collected to determine fresh mass, dry mass (DM), leaf area and chlorophyll concentration.

Shed leaves were collected regularly during the experimental period until November of each year. Collections were made every 3–4 days between August 15 and October 28, 1997 and every 7–10 days between May 27 and November 2, 1998. Leaves remaining on the trees at the time of harvest in early November of each year were detached and collected. The collected leaves were immediately frozen at −20 °C and later thawed, counted and dried at 70 °C to a constant mass. All collected leaves were counted, except for the October 24 and November 2, 1998 collections, when the number of leaves was estimated from the total DM divided by the individual leaf mass in subsamples of 3 × 150 leaves per plot.

Two attached, mature and non-senescent leaves per tree (8–12 leaves per plot) were randomly collected from the mid-canopy on August 12, 1998. All leaves from one plot were combined, dried, weighed and ground in a ball-mill (Retsch type MM2, Retsch, Haan, Germany) for 2 min to obtain a fine powder. Three subsamples (per combined plot sample) of this powder were analyzed for N concentrations with an elemental analyzer (Model: EA 1108 CHNS-O, Fison, Italy) using 2.5-bis-[5-tert-butylenzoxazol-2-yl]-thiophen as a standard. Nitrogen concentrations were also determined for the July 7, August 7 (one subsample per plot), September 10, October 1, 14 and 24, and November 2 (three subsamples per plot) collections of shed leaves.

Calculations and statistics

External ozone exposure was calculated as the exposure above a threshold of 40 nmol mol⁻¹ (AOT40) for 24 h or daylight hours only, whereas the internal ozone exposure was calculated as the cumulative uptake of ozone (CUO) through stomata above a threshold of x nmol m⁻² s⁻¹ (CUO > x; projected leaf area basis). A model parameterized and validated for juvenile B. pendula in southern Sweden was used to model stomatal conductance (Uddling et al. 2004). Maximum stomatal conductance under non-limiting conditions was set to 0.22 mol H₂O m⁻² s⁻¹. Parameters AOT40 and CUO > 1.6 represent the ozone indices for forest trees that are currently being used in the “Critical Levels” concept for plants within the Convention on Long-Range Transboundary Air Pollution pro-
Table 1. Mean ozone concentrations ([O₃]), accumulated O₃ exposure above a threshold of 40 nmol mol⁻¹ calculated for 24 h or daylight hours

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>[O₃](nmol mol⁻¹)</th>
<th>AOT40(µmol mol⁻¹ h)</th>
<th>CUO &gt; x (mmol m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>Daylight</td>
<td>24 h</td>
</tr>
<tr>
<td>1997</td>
<td>NF</td>
<td>22.5</td>
<td>29.3</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>NF+</td>
<td>27.5</td>
<td>36.9</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>NF++</td>
<td>39.0</td>
<td>54.2</td>
<td>36.7</td>
</tr>
<tr>
<td>1998</td>
<td>NF</td>
<td>21.2</td>
<td>24.7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>NF+</td>
<td>32.6</td>
<td>41.6</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>NF++</td>
<td>51.6</td>
<td>70.6</td>
<td>88.2</td>
</tr>
</tbody>
</table>

OZONE IMPAIRS LEAF NITROGEN RESORPTION IN BETULA PENDULA

Results

The mean ozone concentrations and the AOT₄₀, CUO > 1.6 and CUO > 3 values for the two growing seasons are shown in Table 1. Ozone treatments were higher and given during a longer time period in 1998 (April 28 – September 30) than in 1997 (June 4 – September 30), causing the differences in AOT₄₀ and CUO > x between the years to be large in the NF+ and NF++ treatments. Ozone concentrations and AOT₄₀ values were low in NF in both years, as a result of the prevailing northerly winds in 1997 and the cold and rainy weather in 1998.

Net photosynthetic capacity of leaves that emerged in mid-July 1998 declined during August – September in the NF++ treatment, whereas it was stable throughout this period in the NF and NF+ treatments (Figure 1). The NF++ effects were significant from the beginning of September. Elevated ozone caused visible leaf injury (stippling and necroses) and earlier leaf shedding in both years (Figure 2). Tagged leaves that emerged in mid-June 1998 were attached for 128 ± 7, 122 ± 3 and 101 ± 6 days in the NF, NF+ and NF++ treatments, respectively (± SE). In 1998, the accumulated proportions of leaves shed in late June and early October differed significantly among treatments, whereas in 1997 the ozone effect was smaller and only significant in NF++. There were strong linear relationships between the proportion of leaves still attached in early September and October and the daylight AOT₄₀ during the corresponding growing season (Figure 3). The linear regression lines were significantly different from their y-intercepts (daylight AOT₄₀ = 0) at daylight AOT₄₀ > 10 and 7 µmol mol⁻¹ h in September and October, respectively.

Although the total number of leaves and the DM per non-senescent leaf were unaffected by ozone, the DM per shed leaf was higher in NF++ (Table 2). The LMD was significantly lower in NF+ and NF++ than in NF. The ratio of leaf number:total perennial biomass was significantly increased by NF++, reflecting a higher proportional leaf investment in this treatment.

The N concentration of shed leaves in NF and NF+ progressively declined during September and October 1998, whereas it remained high in NF++ during this period (Figure 4). The NF++ effects were significant throughout October, and there was an indication of higher N concentrations in shed leaves in NF+ compared with NF in late October (P = 0.055). Less than...
10% of the total number of leaves in NF and NF+ had leaf N concentrations that were not significantly different from the values for the NF++ plants. The mean N concentration of non-senescent leaves was not significantly affected by ozone, whereas the N concentration of the average shed leaf (including the leaves detached in November) and the total mass of N lost through shed leaves were significantly increased by the NF++ treatment (Table 2). Mean N content per shed leaf increased and NRE decreased with increasing ozone concentrations, with all treatments being significantly different. The reason that total N loss did not differ significantly between the NF and NF+ treatments, in contrast to N content per shed leaf, was the large variation in N loss data. Although N loss is strongly influenced by leaf quantity, it showed a relatively high between-plot variation; N content per shed leaf and NRE are measures of leaf quality and showed low between-plot variation.

Discussion

Ozone was detrimental to _B. pendula_ leaves in this study and all four hypotheses formulated in the introduction were corroborated. Leaves in elevated [O₃] exhibited visible and ultrastructural symptoms of damage (data not shown), and compared with NF leaves, had a lower photosynthetic capacity (Figure 1), a lower N resorption efficiency (Figure 4, Table 2) and were shed earlier (Figures 2 and 3). Nitrogen resorption was more efficient for leaves abscised later in the season in NF and NF+, whereas the timing of leaf abscission seemed to have no effect on N resorption in NF++. Only a small proportion of the leaves in NF and NF+ were shed without an efficient re-

Figure 1. Effect of ozone on net photosynthetic capacity of _Betula pendula_ during 1998 at Östad. Measurements were made on leaves that emerged in mid-July (~ Day 196). Abbreviations: NF = non-filtered air; NF+ = non-filtered air + 10–20 nmol O₃ mol⁻¹ during 0600–1800 h; and NF++ = non-filtered air + 40–60 nmol O₃ mol⁻¹ during 0600–1800 h. An asterisk indicates a significant difference (P < 0.05) between the effects of the NF++ treatment and the effects of the NF and NF+ treatments; the effects of NF and NF+ were not significantly different at any time. Error bars represent SE of the means (n = 4).

Figure 2. Effect of ozone on the accumulation of shed leaves from _Betula pendula_ during 1997 and 1998 in the Östad ozone experiment. Significant (P < 0.05) differences between treatments are denoted by different letters in the order NF, NF+, NF++. (statistical tests were performed for two dates in 1997 and three dates in 1998). Error bars represent SE of the means (n = 4). Abbreviations: NF = non-filtered air; NF+ = non-filtered air + 10–20 nmol O₃ mol⁻¹ during 0600–1800 h; and NF++ = non-filtered air + 40–60 nmol O₃ mol⁻¹ during 0600–1800 h.
causing ozone-induced foliar symptoms in *B. pendula* in Switzerland (~10 µmol mol⁻¹; VanderHeyden et al. 2001). It is also similar to the Critical Level for forest trees currently in force within the Convention on Long-Range Transboundary Air Pollution, United Nations Economic Commission for Europe (10 µmol mol⁻¹; Fuhrer et al. 1997).

Total N loss and mean N content per shed leaf were calculated from measurements of DM and N concentration over the entire 1998 growing season. Maximum N concentration and DM per leaf used in the NRE and LMD calculations were, however, based on small samples of attached, mature and non-senescent leaves in the mid-canopy. The small sample size and the possibility of differences between the selected leaves and the average leaf introduced some uncertainties in the calculated absolute values of NRE and LMD, but does not affect the validity of the relative values. An advantage to expressing the N pool on the basis of leaf units instead of leaf mass or leaf area is that no corrections need to be made for changes in leaf mass:area ratio or leaf shrinkage during senescence in the estimation of NRE (van Heerwaarden et al. 2003). The time between the collections of shed leaves and the assumption that no N was lost through leakage may have caused some over- and underestimation of NRE and N loss, respectively, but the latter effect is expected to be small because N leakage from *B. pendula* is reported to be low (Hagen-Thorn 2004).

Our NRE values are in the upper range of values typically reported for temperate hardwood tree species (Aerts 1996, Duchesne et al. 2001, Côté et al. 2002, García-Plazaola et al. 2003, Hagen-Thorn 2004). In a recent study, *B. pendula* had the highest NRE of four northern deciduous tree species (69%; Hagen-Thorn 2004). The N concentration of shed leaves in NF and NF+ (0.7% at harvest; Figure 4) was similar to values reported for other *Betula* species (Killingbeck 1996), whereas shed leaves in NF++ contained markedly higher N concentrations. Our LMD values are similar to or somewhat higher than values previously reported for other deciduous tree species (van Heerwaarden et al. 2003 and references therein).

There are conflicting reports on how ozone affects N resorption in trees. Ozone is reported to have no effect on NRE in the

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**Table 2. Effects of ozone on leaf number, mean dry mass and nitrogen (N) concentration of green¹ and shed² *Betula pendula* leaves during the 1998 growing season in the Östad ozone experiment. Also shown are the effects of ozone on N content per leaf, N resorption efficiency (NRE), leaf dry mass decrease (LMD) and total N loss per tree, as well as the number of leaves per perennial biomass. Abbreviations: NF = non-filtered air; NF+ = non-filtered air + 10–20 nmol O₃ mol⁻¹ during 0600–1800 h; and NF++ = non-filtered air + 40–60 nmol O₃ mol⁻¹ during 0600–1800 h. Treatment means are shown ± SE (*n* = 4 = number of plots per treatment). Treatments that were significantly different (*P* < 0.05) are denoted by different letters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Leaf</th>
<th>Ozone treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF</td>
<td>NF+</td>
</tr>
<tr>
<td>No. of leaves per tree</td>
<td>3368 ± 140 a</td>
<td>3388 ± 194 a</td>
</tr>
<tr>
<td>No. of leaves per perennial dry mass (g⁻¹)</td>
<td>1.94 ± 0.09 a</td>
<td>2.09 ± 0.15 a</td>
</tr>
<tr>
<td>Dry mass per leaf (mg)</td>
<td>119 ± 4 a</td>
<td>110 ± 6 a</td>
</tr>
<tr>
<td></td>
<td>66 ± 3 a</td>
<td>70 ± 2 a</td>
</tr>
<tr>
<td>N concentration (% of dry mass)</td>
<td>2.48 ± 0.12 a</td>
<td>2.31 ± 0.10 a</td>
</tr>
<tr>
<td></td>
<td>0.80 ± 0.03 a</td>
<td>0.87 ± 0.04 a</td>
</tr>
<tr>
<td>N content per leaf (mg)</td>
<td>2.93 ± 0.11 a</td>
<td>2.54 ± 0.12 a</td>
</tr>
<tr>
<td></td>
<td>0.52 ± 0.01 a</td>
<td>0.61 ± 0.02 b</td>
</tr>
<tr>
<td>NRE (%)</td>
<td>82 ± 1 a</td>
<td>76 ± 1 b</td>
</tr>
<tr>
<td>LMD (%)</td>
<td>45 ± 1 a</td>
<td>36 ± 2 b</td>
</tr>
<tr>
<td>Total N loss per tree (g)</td>
<td>1.76 ± 0.07 a</td>
<td>2.05 ± 0.13 a</td>
</tr>
</tbody>
</table>

¹ Attached, mature, green and non-senescent leaves sampled in August.
² Leaves collected as litter or detached at plant harvest on November 2, 1998.
The ozone-induced decrease in NRE in *B. papulifera* L. (Scherzer et al. 1998) and species, ozone had no effect on N resorption in the presence of ozone (Temple and Riechers 1995). For deciduous tree species, ozone had no effect on N resorption in *Liriodendron tulipifera* L. (Scherzer et al. 1998) and *Populus tremuloides* Michx. (Lindroth et al. 2001), whereas NRE was significantly decreased by ozone in *Betula papyrifera* Marsh. (Lindroth et al. 2001). The ozone-induced decrease in NRE in *B. papyrifera* was ameliorated in air enriched in CO₂. In *Populus tremula* L., leaves showing bronzing in the presence of an elevated concentration of ozone were shed without yellowing (Matyssek et al. 1993), whereas in open-soil-grown *B. pendula*, N resorption from attached leaves during September was increased by ozone (Oksanen 2003). Nitrogen resorption was very inefficient in *Populus x euramericana* in an elevated concentration of ozone; N concentrations in shed foliage were at least 70% of the concentrations in attached leaves (Schmutz et al. 1995). Exposure of *Populus deltoids* Bartr. ex Marsh. to 200 nmol mol⁻¹ ozone for 5 h was enough to cause premature leaf abscission of leaves with increased concentrations of N (Findlay et al. 1996). Despite having higher N concentrations, these leaves had lower rates of decomposition caused, it has been suggested, by increased concentrations of complex and less easily extractable phenolics.

There are several possible explanations for the ozone-induced inhibition of N resorption that we observed. The lower photosynthetic capacity of old leaves in the NF++ treatments may have impaired N resorption, because it appears likely that the resorption is dependent on a competent photosynthetic system able to supply the energy needed for catabolic activity and to drive the export of photosynthate from the leaves through the phloem. Leaf shading (Chapin and Moilanen 1991, May and Killingbeck 1992) as well as catkin (a carbon sink) removal (Chapin and Moilanen 1991) have both been shown to decrease NRE in deciduous woody plant species. Furthermore, it is possible that, in our study, ozone impaired the process of phloem-loading in leaves, because deformed phloem tissue and accumulated starch along leaf veins have been observed in *B. pendula* in response to elevated concentrations of ozone (Günthardt-Goerg et al. 1993). Impaired phloem-loading may result from cell membrane damage in the mesophyll cells, which is a typical effect of ozone (Heath 1987). Ozone can also cause several other structural and biochemical changes that differ from normal leaf senescence changes (for birch: Günthardt-Goerg et al. 1993, 1997, Piäkkönen et al. 1995), several of which may have caused the impaired N resorption that we observed. For example, ozone can trigger a hypersensitive response considered to be a form of programmed cell death (Rao and Davis 2001). Rapid apoptosis-like programmed cell death frequently prevents the complete degradation of cell organelles (Fukuda 2000), leaving dead and collapsed mesophyll cells of leaves exhibiting visible ozone symptoms with remnants of nuclei and chloroplasts (Vollenweider et al. 2003). Leaves in the NF++ and, to a lesser extent, the NF+ treatments exhibited stippling and necroses, and it is possible that the disturbed resorption from these leaves was caused by ozone-induced rapid cell death.

Nitrogen resorption before leaf abscission is a major N conservation mechanism in deciduous trees, increasing growth and making it less temporarily dependent on current root uptake (Aerts 1996). An ozone-induced decrease in NRE can be expected to have a negative impact on growth and fitness. It appears likely that some of the carry-over effects previously reported for *B. pendula* (e.g., reduced relative growth rate and decreased leaf concentrations of nutrients, chlorophyll and Rubisco; Oksanen and Saleem 1999) were at least partly caused by impaired resorption of nutrients from the leaves before abscission in preceding year(s). Increased N loss through litter fall in response to elevated concentrations of ozone may be particularly serious, as another common effect of ozone is a reduced root:shoot ratio (–27% in this study; Karlsson et al. 2003).

In summary, we conclude that ozone impaired the autumnal resorption of N from birch leaves, causing a substantial increase in whole-tree N loss. Several ozone-induced changes in leaf structure and physiology observed in studies with *B. pendula* provide possible explanations for this effect, which may have important implications for tree growth and fitness in the field.

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