



Commentary

Enhanced ozone strongly reduces carbon sink strength of adult beech (*Fagus sylvatica*) – Resume from the free-air fumigation study at Kranzberg Forest

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Empirical proof corroborates substantial mitigation of carbon sequestration in the tree–soil system of a forest site under enhanced O₃ impact for adult beech.

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ABSTRACT

Ground-level ozone (O₃) has gained awareness as an agent of climate change. In this respect, key results are comprehended from a unique 8-year free-air O₃-fumigation experiment, conducted on adult beech (*Fagus sylvatica*) at Kranzberg Forest (Germany). A novel canopy O₃ exposure methodology was employed that allowed whole-tree assessment *in situ* under twice-ambient O₃ levels. Elevated O₃ significantly weakened the C sink strength of the tree–soil system as evidenced by lowered photosynthesis and 44% reduction in whole-stem growth, but increased soil respiration. Associated effects in leaves and roots at the gene, cell and organ level varied from year to year, with drought being a crucial determinant of O₃ responsiveness. Regarding adult individuals of a late-successional tree species, empirical proof is provided first time in relation to recent modelling predictions that enhanced ground-level O₃ can substantially mitigate the C sequestration of forests in view of climate change.

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1. Introduction

Ground-level ozone (O₃), if occurring at concentrations above the pre-industrial range, is potentially the most detrimental of all air pollutants for vegetation (Reich and Amundson, 1985;

Bytnerowicz et al., 2003), including trees and forests (Matyssek and Sandermann, 2003). O₃ levels are predicted to stay high globally or increase, particularly in rapidly developing countries (Fowler et al., 1999), and O₃ must be included in climate change scenarios and post-Kyoto policies (Ashmore, 2005). Combined free-air carbon dioxide and O₃-enrichment studies show that O₃ reduces carbon (C) sequestration, especially in fast-growing pioneer tree species (Matyssek et al., 2010). Incorporating this evidence in models led Sitch et al. (2007) to predict that O₃ will promote radiative forcing *via* substantial C sink strength limitation. However, there were no reliable data for O₃ effects on naturally growing adult forest trees, especially late-successional species.

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¹ Dedication: To our late colleague and friend, Heinrich Sandermann, who was involved in initiating and preparing this joint manuscript, but to whom it was not granted to see its completion.

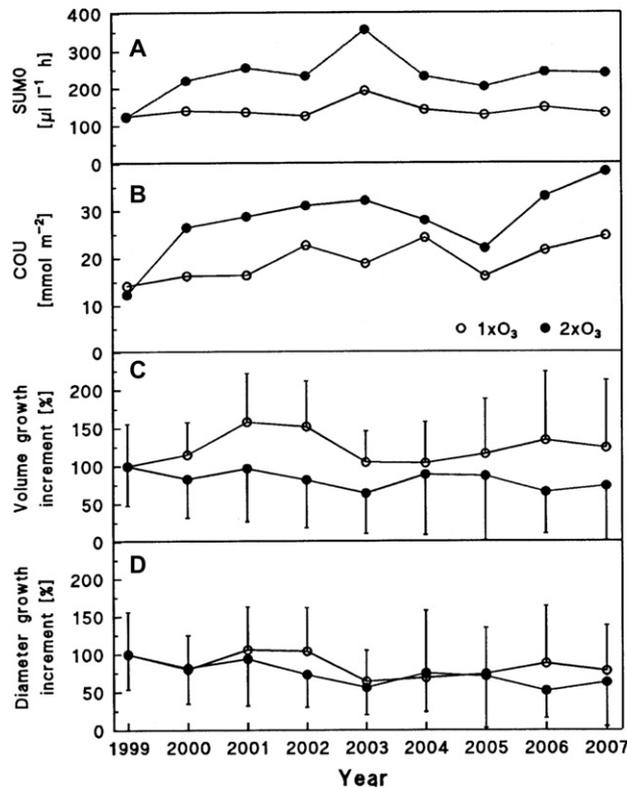


Fig. 1. Temporal change in ozone exposure and uptake along with radial and whole-stem volume increment. A, cumulative O₃ exposure (SUMO), B, cumulative O₃ uptake (COU) measured 20 m above ground within the canopy during growing seasons; C, relative annual increase in radial stem growth at breast height of adult beech (*Fagus sylvatica*) trees, as related to mean increment of 1994 through 1999 (set to 100%; cf. Wipfler et al., 2005). D, annual whole-stem increment based on the allometry of the stem height–diameter relationship (cf. Pretzsch et al., 2010). 1×O₃ (open symbols) or experimentally enhanced 2×O₃ (solid symbols) during the years 1999 through 2007. Data are means of 5 trees ± standard deviation each.

Results to date have typically been from juvenile trees in closed chambers, and therefore are biased by the unnatural growth conditions and altered plant sensitivity to O₃ (Kolb and Matyssek, 2001; Stockwell et al., 1997), so that value for extrapolation to forests in the real world is limited. Here, we comprehend the key results from a unique 8-year free-air O₃-fumigation experiment on adult beech (*Fagus sylvatica*), an ecologically and economically important climax tree in Central Europe, conducted at Kranzberg Forest (Germany; Matyssek et al., 2007a). A novel canopy O₃ exposure methodology was employed that allowed whole-plant assessment of *in situ* growing forest trees under twice-ambient O₃ levels (2×O₃; Werner and Fabian, 2002). The 2×O₃ regime was based on monitoring the ambient O₃ regime at the site (1×O₃) continuously throughout eight growing seasons (2000 through 2007), with trees under 1×O₃ as reference. The extent will be highlighted to which, over the study period, elevated O₃ affected C sink strength, along with responses in leaves and roots at the gene, cell and organ levels. Attention will be directed, in addition, to drought as a crucial determinant of O₃ responsiveness (Löw et al., 2006; Nikolova et al., 2010), and as to whether consistency exists under prevalent stand conditions with recent modelling predictions about chronic O₃ impact on forest productivity (Sitch et al., 2007).

2. Experimental set-up, site conditions and O₃ regimes

The study site Kranzberg Forest (mixed *Fagus sylvatica* L./*Picea abies* [L.] Karst. forest) was located near Freising/Germany at 48°25′08″N and 11°39′41″E (485 m a.s.l.). Trees were 60 years old and up to 28 m high in a closed canopy (leaf area index of 5.4, stem production of 23.5 m³ ha⁻¹ a⁻¹; details see Pretzsch et al., 1998). Tree crowns were accessed via scaffolding and a research crane. Long-term (1970–2000) averages of annual mean air temperature and precipitation were 7.8 °C and 786 mm, respectively (Deutscher Wetterdienst, DWD, station “Weihenstephan”). Air temperature was >10 °C, on average, during 155 days of the year. The year 2003 was exceptional during the study period of 1999 through 2007: average air temperature in summer was increased from 14.8 to 18 °C, while precipitation was reduced by 37% relative to the long-term average. The free-air canopy O₃-fumigation system consisted of 150 teflon tubes vertically suspended, at 0.5 m distances, across the foliated canopy and releasing O₃ through pressure-calibrated capillary outlets at 0.3 m intervals (Werner and Fabian, 2002). A volume of 2000 m³, comprising five adjacent beech and spruce trees each was exposed to 2×O₃ (relative to the unchanged 1×O₃ regime at the site). As no tree enclosure was used, there was no micro-climatic bias on O₃ sensitivity. While 2×O₃ was restricted to <150 nl l⁻¹ to prevent acute injury, a corresponding number of trees served as reference under 1×O₃. Each individual tree was regarded as

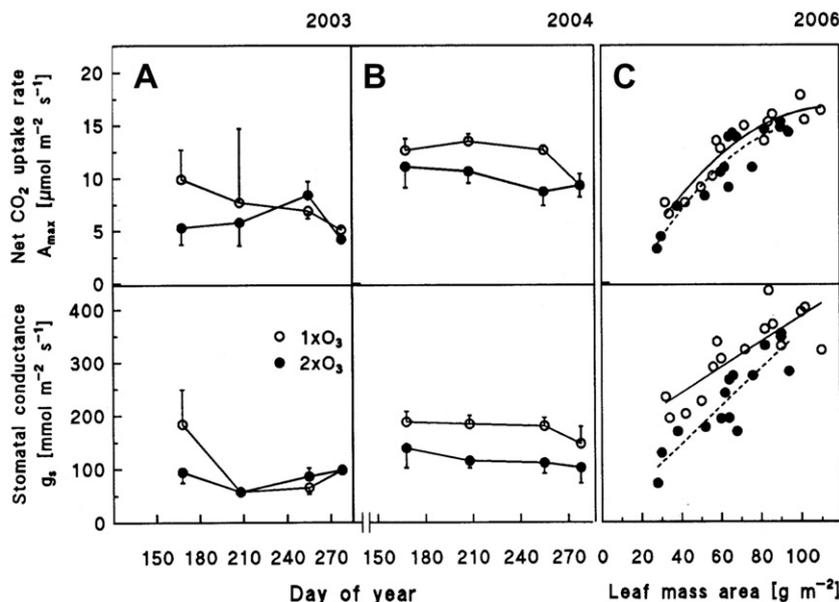


Fig. 2. Relationship between net photosynthetic rate (upper graph) and stomatal conductance for water vapour (lower graph) to (A, B) day of year during 2003 (dry growing season) and 2004 (humid growing season, Löw et al., 2006) and (C) the leaf mass per area ratio of leaves (during 2006, Kitao et al., 2009, humid growing season).

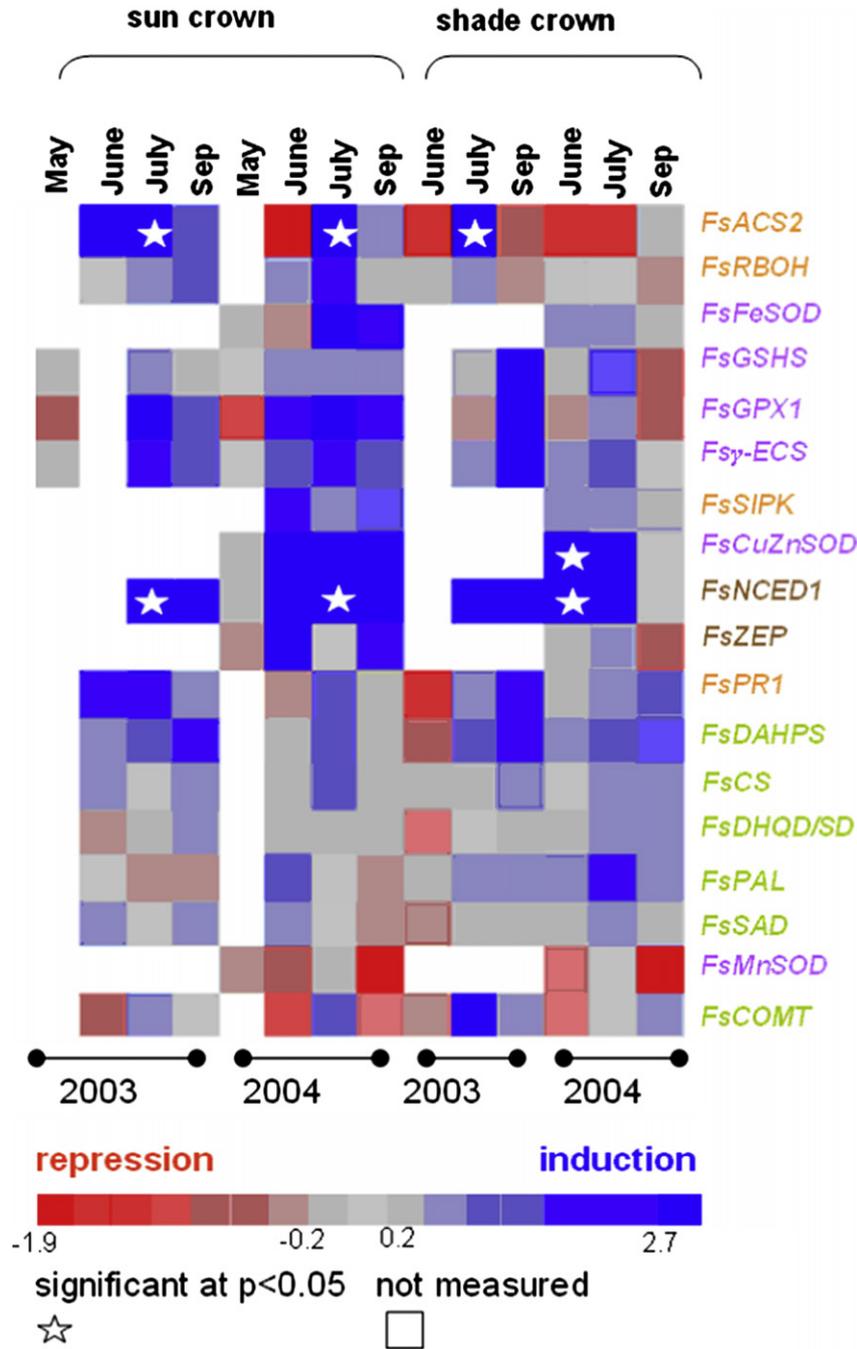


Fig. 3. Patterns of gene expression in leaves. Data show colour-coded log ratios of gene expression under $2 \times O_3$ in relation to $1 \times O_3$ in 2003 and 2004 (modified from Jehnes et al. 2007, *Plant Biol.* 9, 253–264). Each gene is represented by a row, light sun/shade crown exposure and sampling dates by a column. Text colours of genes indicate their affiliation with physiological functions involved in ozone defence. Orange: Programmed cell death (PCD, ROS production); *FsACS2*, 1-aminocyclopropane-1-carboxylic acid synthase; *FsRBOH*, respiratory burst oxidase; *FsSIPK*, salicylic acid induced mitogen-activated protein kinase; *FsPRI*, pathogenesis-related protein, violet: antioxidative response (*FsGSHS*, glutathione synthase; *FsGPX1*, glutathione peroxidase; *Fsγ-ECS*, γ-glutamylcysteine synthetase; *FsFeSOD*, iron superoxide dismutase; *FsCuZnSOD*, copper/zinc superoxide dismutase; *FsMnSOD*, manganese superoxide dismutase); brown: stomatal closure (*FsNCED1*, 9-cis-epoxycarotenoid dioxygenase; *FsZEP*, zeaxanthin epoxidase); green: lignin synthesis (*FsCOMT*, caffeic acid O-methyl transferase; *FsDAHPS*, 3-deoxy- β -arabino-heptulosonic-acid-7-phosphate-synthase; *FsCS*, chorismate synthase; *FsDHQD/SD*, 5-dehydroquinate-dehydratase/shikimate dehydrogenase; *FsPAL*, phenylalanine ammonia lyase; *FsSAD*, sinapoyl-alcohol dehydrogenase). The array has been generated using the Cluster and Tree View software (M. Eisen, Stanford University/USA).

a replicated case study of its own (Matyssek, 2007). The experiment was operated continuously during the growing seasons of the 8-year study period. Details of the O_3 -fumigation system are published (Werner and Fabian, 2002), and methods employed during molecular, biochemical and (eco-) physiological investigations were addressed in Matyssek (2007) and additional references provided therein.

The local $1 \times O_3$ regime at the forest site, expressed as the cumulative O_3 exposure (SUM0), varied over each growing season around $140 \mu l l^{-1} h$ (Fig. 1A), except for the extraordinarily sunny and dry summer of 2003 (Ciais et al., 2005). SUM0, as an external O_3 index, was close to double under the $2 \times O_3$ regime. Stomatal closure in

beech prevented enhanced O_3 influx into leaves during the drought of 2003 (at external SUM0 of $194 \mu l l^{-1} h$ at $1 \times O_3$). As a result, the difference between the two O_3 regimes in cumulative O_3 uptake (COU, Fig. 1B) was less than the difference in SUM0.

3. Mitigation of carbon sequestration in the tree–soil system

Whole-stem volume increments revealed the deleterious effect of $2 \times O_3$ in beech throughout the study period (Pretzsch et al., 2010;

Fig. 1 C). This result apparently contradicts previous reports from the same beech trees stating that ozone did not significantly affect annual stem increment (Wipfler et al., 2005). The previous analysis was based, however, on radial growth at breast height in accordance with conventional forestry practices (Fig. 1 D). The data has now been re-analysed using a different measurement of growth, i.e. based on the diameter-height allometric relationship, which characterizes individual stem shapes along the entire stem length (Pretzsch et al., 2010). At stand level, the decline in stem productivity attributable to O_3 was $10 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$, on average, during the 8-year study period, representing a 44% loss relative to the $1 \times O_3$ trees. Such an effect was absent in spruce, where O_3 -changed diameter allometry in the upper stem compensated for a minor reduction in radial increment at breast height (Wipfler et al., 2005). Overall, spruce displayed increase by $0.5 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ under $2 \times O_3$ (Pretzsch et al., 2010).

The measured decline in the stem growth of beech validates predictions from modelling that elevated O_3 will result in a substantial reduction of C sink strength in trees (Sitch et al., 2007). The used C cycling model relied on data from juvenile trees (Karlsson et al., 2004) and crops (Pleijel et al., 2004) in chambers, conditions favouring O_3 uptake (with tree modelling validated through Aspen FACE poplar data; Karnosky et al., 2003). Our empirical study demonstrates for the first time that whole-stem growth of adult trees of a climax species under natural stand conditions is restricted under enhanced O_3 impact.

In contrast to most other studies, elevated O_3 did not affect mesophyll photosynthesis of beech directly, but reduced CO_2 assimilation by stomatal closure in both sun and shade foliage (Kitao et al., 2009; Fig. 2 C). In humid years, when $2 \times O_3$ restricted photosynthesis seasonally (Fig. 2 B, similar to e.g. 2006 shown in Fig. 2 C), stomatal closure was linked to increased photosynthetic water-use efficiency and $^{13}C/^{12}C$ ratios irrespective of leaf type (Kitao et al., 2009) and, at times, lowered leaf contents of starch and sucrose (Blumenröther et al., 2007). O_3 -induced stomatal closure was associated with the induction of the ABA biosynthesis gene *NCED1*, whose transcripts were more abundant in $2 \times O_3$. No consistent differences in expression were detected for other genes (Fig. 3) but at periods of exceptionally high O_3 levels there were indications of oxidative stress (Matyssek and Sandermann, 2003, induction of salicylic acid and lignin formation), response to injury (e.g. via 1-aminocyclopropane-1-carboxylic acid, the ethylene precursor), and challenge to plant defences (expression of shikimate and glutathione pathways at reduced levels of glutathione and total ascorbate). The lowered starch and sucrose levels (Blumenröther et al., 2007) appear to result from increased glycolysis (Einig et al., 1997), consistent with enhanced leaf dark respiration (Kitao et al., 2009), to fuel these responses and repair the injury caused by $2 \times O_3$. Nevertheless, in humid years plant counter-measures did not entirely prevent minor macroscopic leaf injuries, shortening of the growing season (by up to 10 days) as a result of delayed leaf flush, and accelerated senescence (Nunn et al., 2005a). Added to enhanced respiration and stomatal limitation of photosynthesis, these deleterious effects of elevated O_3 further curtailed the C gain available for stem growth. Also in spruce, photosynthetic limitation occurred in humid years under $2 \times O_3$ at reduced stomatal conductance, although such effects were mainly restricted to shade crowns (Nunn, 2005; Nunn et al., 2005b).

Although O_3 does not physically penetrate soil, the sink strength for carbon was curtailed in $2 \times O_3$ not only above ground in the stem, but also below-ground, as a result of a tendency of reduced C allocation (Andersen et al., in press) but increased soil respiration (Nikolova et al., 2010) in both beech and spruce during humid years (Fig. 4). Hence, in beech the carbon storage capacity was reduced in the entire tree–soil system. The respiratory effect was accompanied

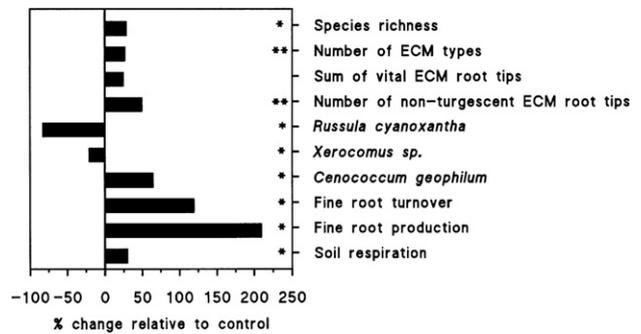


Fig. 4. Effects of ozone on below-ground ecosystem components. Percent change in mycorrhizal characteristics (species richness, number of ECM types, sum of vital and number of non turgescient ECM root tips, abundance of *Russula cyanoxantha*, *Xerocomus sp.* and *Cenococcum geophilum*; Grebenc and Kraigher, 2007; Haberer et al., 2007) and annual fine-root turnover, fine-root production and soil respiration rate (absolute units were $[a^{-1}]$, $[g \text{ m}^{-2} \text{ a}^{-1}]$ and $[g \text{ CO}_2 \text{ m}^{-2} \text{ h}^{-1}]$, respectively) under *Fagus sylvatica* trees exposed to $2 \times O_3$ under humid summer conditions in relation to the respective parameter under $1 \times O_3$ (=0%). Each value represents the mean of 5 assessments. Statistically significant at $p < 0.05$ (*) and $p < 0.01$ (**).

in beech by increased diversity of mycorrhizal associations and enhanced annual production of fine roots under $2 \times O_3$ (Nikolova et al., 2010; Grebenc and Kraigher, 2007; Haberer et al., 2007; Fig. 4). The promotion of fine-root growth in response to O_3 stress is in marked contrast to the reduction in root growth – due to disruption of phloem translocation of C – typically reported for elevated O_3 from chamber studies (Matyssek and Sandermann, 2003). The fine-root stimulation points to a role for phytohormones, in particular cytokinins (Winwood et al., 2007), in communicating the impact of O_3 above ground down to the roots below. O_3 -mediated destruction of cytokinins in leaves led to declining retranslocation from leaves via the phloem to the root system – a change known to relieve inhibition of fine-root production (Riefler et al., 2006). Crown-to-root signalling of ozone effects is further supported by the finding that ozone exposure modified root glutathione concentrations (Haberer et al., 2008). Glutathione is known to affect dividing cells in root meristems (Zellnig et al., 2000), therefore potentially contributing to changes in fine roots.

4. Drought – a crucial modifier of O_3 impact

In the dry year of 2003 (Ciais et al., 2005), prolonged water shortage rather than O_3 stress limited both the radial and whole-stem volume increment of the beech trees (Fig. 1C, D). Drought also nullified the O_3 -driven stimulation of below-ground activity (Nikolova et al., 2010, 2009). At the leaf level, the impact of O_3 was reduced because from early summer drought-driven stomatal closure pre-empted O_3 -driven closure (Löw et al., 2006; Fig. 2 A). As a result, seasonal O_3 uptake in 2003 was slightly less than the average for the humid years, even though O_3 exposure was greater by 41% (Fig. 1 A, B) – drought actually decoupled O_3 uptake from O_3 exposure. Apparently, the assumption on which current protection policy in Europe is based, that O_3 exposure correlates reliably with O_3 uptake, was not substantiated (cf. Wieser and Tausz, 2007).

5. Conclusions

Our findings corroborate the high sensitivity of stem growth to O_3 reported from studies of juvenile, mostly potted trees (including beech) in chambers (Kolb and Matyssek, 2001), although the nature of the response suggests the underlying mechanisms to differ both qualitatively and quantitatively. By contrast with beech, however, adult Norway spruce (*Picea abies*), also shade-tolerant and late-

successional, at the same site did not display decline in whole-stem productivity (Pretzsch et al., 2010), although the conventional analysis of radial growth at breast height had indicated an inhibitory effect of $2 \times O_3$ (Wipfler et al., 2005). Our findings from beech and spruce, therefore, suggest that the most commonly used stem growth assessment method is not sufficient to assess the impact of environmental stress. Also, our study underlines the need to understand the O_3 risks for individual species when modelling tree and stand performance in climate change. In addition, the current impetus in Europe to change O_3 risk assessment from exposure-based to flux-based concepts (Matyssek et al., 2007b) is justified by our study. Drought, and other variable stresses that interfere with metabolism and stomatal regulation (Matyssek et al., 2008), will complicate O_3 risk modelling and long-term prognoses by altering the relationship between external and internal exposure. In parallel to the significantly reduced whole-stem growth of adult beech under $2 \times O_3$, the spectrum of further O_3 responses reflected O_3 stress to be metabolically experienced by the trees. In the long term, therefore, weakening of this important forest tree species in Central Europe by exposure to ground-level ozone apparently becomes a realistic scenario.

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