Review

Ozone induces stomatal narrowing in European and Siebold's beeches: A comparison between two experiments of free-air ozone exposure

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Abstract

Stomata tend to narrow under ozone (O3) impact, leading to limitation of stomatal O3 influx. Here, we review stomatal response under recently conducted free-air O3 exposure experiments on two species of the same tree genus: Fagus sylvatica at Kranzberg Forest (Germany) and F. crenata at Sapporo Experimental Forest (Japan). Both beeches exhibited reduction in stomatal conductance (gs) by 10–20% under experimentally enhanced O3 regimes throughout the summer relative to ambient-air controls. Stomatal narrowing occurred, in early summer, in the absence of reduced carboxylation capacity of Rubisco, although photosynthetic net CO2 uptake rate temporarily reflected restriction to some minor extent. Observed stomatal narrowing was, however, diminished in autumn, suggesting gradual loss of stomatal regulation by O3. Monotonic decline in gs with cumulative O3 exposure or flux in current modeling concepts appear to be unrealistic in beech.

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1. Introduction

Ground level ozone (O3) concentration has doubled to date in the northern hemisphere since pre-industrial times, while as depending on region continued enhancement or further increase are prognosticated globally throughout the upcoming decades (Akimoto, 2003; Vingarzan, 2004; Sitch et al., 2007). Ozone is recognized as a significant phytotoxic air pollutant to cause adverse effects on forest ecosystems (Karnosky et al., 2003; Matyssek and Sandermann, 2003; Ashmore, 2005; Bytnerowicz et al., 2007; Serengil et al., 2011; Matyssek et al., 2010a, 2014b).

Fagus (beech) is a widespread genus of ecologically and economically important deciduous tree species across Europe, Asia and North America. European beech (Fagus sylvatica) and Siebold’s beech (Fagus crenata), being dominant beeches in Europe and Japan, respectively. Both species are distributed in temperate climate regions. European beech is found even in dry climate (southern Italy, ~37.7 °C14N) whereas Siebold’s beech is confined to humid climate (Fang and Lechowicz, 2006). These two species have been experimentally investigated in view of their O3 sensitivities previously under chamber conditions (Grams et al., 1999; Kozovits et al., 2005a,b; Karlsson et al., 2007; Pritsch et al., 2005; Grams and Matyssek, 2010; Luedemann et al., 2005, 2009; Yamaguchi et al., 2011). As a result, both species have been classified as O3 sensitive (Kohno et al., 2005; Karlsson et al., 2007; Mills et al., 2010).

O3 impact is mediated through stomatal O3 flux (Omasa et al., 2002; Karlsson et al., 2007; Matyssek et al., 2007a, 2008; Mills et al., 2010), because stomata are the principal interface for entry of O3 into plants (cf. Wittmann et al., 2007). A meta-analytic review showed that stomatal narrowing was generally caused by O3 impact, hence, leading to reduce stomatal O3 influx (Wittig et al., 2007). Modeling studies for assessment of O3 impacts in forest trees strive for integrating empirical knowledge through considering O3-induced stomatal narrowing, assuming that stomatal

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conductance decreases monotonically with cumulative \( O_3 \) exposure or flux (e.g., Felzer et al., 2004; Sitch et al., 2007; Collins et al., 2010; Mills et al., 2010).

Most of the information regarding \( O_3 \) effects on trees, however, has been derived from chamber experiments (Pearson and Mansfield, 1993; Le Thiec et al., 1994; Grams et al., 1999; Bortier et al., 2000; Matyssek and Innes, 1999; Watanabe et al., 2005; Gerost et al., 2008; Matyssek et al., 2010a; Watanabe et al., 2010). Particular meteorological conditions in chambers (e.g., enhanced air temperature, high air turbulence) can change plant response to \( O_3 \) relative to actual field conditions (Nussbaum and Fuhrer, 2000). Hence, available knowledge and hypotheses have to be evaluated under ecologically relevant forest site conditions, e.g. through technologies such as the recently developed experimental free-air canopy \( O_3 \) exposure approach (Matyssek et al., 2010a), being focused on forest trees in comparison with a limited number of similar experiments conducted around the world (Karnosky et al., 2007; Matyssek et al., 2010b; Watanabe et al., 2013).

In addition to the different site conditions, tree ages differ between free-air \( O_3 \) exposure experiments. Difference in the response to \( O_3 \) between different aged trees has been discussed in several studies (Kolb et al., 1997; Kolb and Matyssek, 2001; Matyssek and Sandermann, 2003). Matyssek et al. (2010a) concluded that adult and juvenile trees of pioneer and climax tree species show similar sensitivity of tree growth to chronic \( O_3 \) stress, and, however, suggested that the response mechanisms may differ. In general, some leaf physiological parameters are known to change with tree size and age (e.g., Steppe et al., 2011). For instance, stomatal conductance declines with advancing tree dimension and age due to hydraulic constraints (Schäfer et al., 2000; Ryan et al., 2006; Nabeshima and Hiura, 2008), although adverse effects stayed absent on photosynthetic capacity in aging Siebold’s beech (Koike, 1988) and adult European beech (Herbinger et al., 2005; Häberle et al., 2012). Experimental examination is advocated, therefore about the ways \( O_3 \) may affect stomatal regulation in aging forest trees for consolidating ecologically meaningful knowledge about \( O_3 \) impacts throughout tree ontogeny. Evidential consistency may infer generisec in tree response to chronic \( O_3 \) stress. Absence of consistency would imply species-driven \( O_3 \) response, notwithstanding potential effects e.g. by ontogenetic stage, tree dimension or site conditions.

Here, we exemplify a comparison within \( Fagus \) between the two above-mentioned species as growing either in Germany (European beech, Kranzberg Forest) or in Japan (Siebold’s beech, Sapporo Experimental Forest). At both locations, free-air canopy \( O_3 \) exposure experiments were operated, consistently employing the methodology as described by Nunn et al. (2002), Werner and Fabian (2002) and Karnosky et al. (2007). In both cases, \( O_3 \) effects on stomatal conductance were of interest (Matyssek et al., 2010b; Watanabe et al., 2013). Humid years were selected for the comparison, excluding drought effects on stomata from the analysis. Assessment of stomatal conductance was performed during 09:00 through 15:00 h at both sites, when photosynthetic activity was stable (no midday depression). A differential evaluation will be presented, concluding about regulatory mechanisms that may underlie stomatal responsiveness in either case. The envisaged clarification is intended to consolidate risk assessment and providing grounds for cause-effect based modeling of \( O_3 \) impacts on forest trees.

2. Free-air ozone exposure experiments on beech trees

2.1. Kranzberg Forest

The mixed \( Fagus sylvatica \) L./\( Picea abies \) [L.] Karst. stand was part of Kranzberg Forest, located near Freising, southern Germany, in the vicinity of Munich (48°25'08" N, 11°39'41" E, 485 m a.s.l., annual mean temperature and precipitation: 8.8 °C and 698 mm, respectively, in 2006; see Table 1). Ambient \( O_3 \) concentration was around 385 nmol mol\(^{-1}\) (Grams et al., 2011). The soil at the site was a luvisol derived from loess over Tertiary sediments. The European beech trees were about 80 years old and up to 28 m high at the time of assessment (Pretzsch et al., 1998, 2010; Reiter et al., 2005; Häberle et al., 2012, Table 1). A novel free-air \( O_3 \) fumigation system (Nunn et al., 2002; Werner and Fabian, 2002; Karnosky et al., 2007) was employed throughout growing season of 2000 through 2007 to experimentally expose five adjacent trees of European beech to a \( 2 \times \) ambient \( O_3 \) regime (\( 2 \times O_3 \) as elevated \( O_3 \)). To prevent acute \( O_3 \) injury, the regime was restricted in order to avoid exceeding 150 nmol \( O_3 \) mol\(^{-1}\) (Fig. 1). Other five adjacent European beech trees under the unchanged \( O_3 \) regime of the ambient air served as control (\( 1 \times O_3 \); Nunn et al., 2002; Matyssek et al., 2007b). The daytime hourly mean \( O_3 \) concentration during the growing season in 2006 was 36 nmol mol\(^{-1}\) and 66 nmol mol\(^{-1}\) under \( 1 \times O_3 \) and \( 2 \times O_3 \), respectively (Fig. 1; Table 1).

2.2. Sapporo Experimental Forest

Sapporo Experimental Forest of Hokkaido University is located in northern Japan (43°04′ N, 141°20′ E, 15 m a.s.l., annual mean temperature: 9.3 °C, total precipitation: 1279 mm in 2012; see Table 1) on a brown forest soil. Ambient \( O_3 \) concentration was around 390 nmol mol\(^{-1}\) (Watanabe et al. person. comm.). Siebold’s beech (\( Fagus crenata \)) saplings grown at the site in 2012 were 11 years old and 3.4 m in height. The employed experimental free-air \( O_3 \) exposure system was based on the methodology and design used at Kranzberg Forest (details see Watanabe et al., 2013), with one plot exposed to unchanged ambient air (control), and the other one to the experimentally enhanced \( O_3 \) regime (Fig. 1, Table 1; target level of 60 nmol \( O_3 \) mol\(^{-1}\) during daylight hours). \( O_3 \) enhanced was employed during daytime to ten Siebold’s beech saplings from August through November in 2011, and from May through November in 2012. Daytime hourly mean \( O_3 \) concentration in control and elevated \( O_3 \) were 28 nmol mol\(^{-1}\) and 62 nmol mol\(^{-1}\), respectively, during the experimental period in 2012 (Fig. 1, Table 1). Hence, mean seasonal \( O_3 \) concentrations were similar in both experiments, although higher peak \( O_3 \) concentrations occurred at Kranzberg Forest.

3. Ozone effects on stomatal conductance of European and Siebold’s beech in free-air ozone exposure experiments

3.1. Reduced stomatal conductance under enhanced \( O_3 \) stress

Leaf mass per area (LMA) was used as an index of light environment for each assessed leaf regardless of \( O_3 \) treatments, yielding

<table>
<thead>
<tr>
<th>Species</th>
<th>Kranzberg forest</th>
<th>Sapporo experimental forest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree age (years old)</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Tree height (m)</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Mean annual temperature (°C)</td>
<td>8.8b</td>
<td>9.3a</td>
</tr>
<tr>
<td>Annual precipitation (mm)</td>
<td>698b</td>
<td>1279b</td>
</tr>
<tr>
<td>Mean hourly ( O_3 ) concentration during daytime (nmol mol(^{-1}))</td>
<td>36c</td>
<td>28c</td>
</tr>
</tbody>
</table>

a Obtained in the year 2006.  
b Obtained in the year 2012.
no difference in LMA between ambient and elevated O₃ regimes both in European and Siebold’s beech (Kitao et al., 2009; Watanabe et al., 2013, 2014). Leaf gas exchange along the within-canopy light gradient revealed reduced stomatal aperture under enhanced O₃ stress in trees of European and Siebold’s beech (Kitao et al., 2009; Watanabe et al., 2014, Fig. 2). In such terms, stomatal response of both beech species to O₃ did not differ between the study sites, although difference existed when relating stomatal behavior to respective tree age. Tree age can be a determinant of stomatal conductance (Kolb and Matyssek, 2001). However, reduction of stomatal conductance by O₃ on both sites followed similar seasonal trends (Fig. 2). Accordingly, sun and shade leaves consistently showed reduction in stomatal conductance under elevated O₃ in both experiments. Stomatal conductance declined under elevated O₃ by 20% in sun leaves and 12% in shade leaves of European beech at Kranzberg Forest relative to the control (Nunn et al., 2005). Decline could reach about 33% during humid years in sun leaves, although the O₃ effect was overwhelmed by the stomata-closing impact of severe drought (Löw et al., 2006). Similar extents of limitation in stomatal conductance caused by elevated O₃ were found in Siebold’s beech in Japan (Hoshika et al., 2012b, unpublished), being 20% and 12% in sun leaves during 2011 and 2012, respectively, and 15% in shade leaves during 2012 in relation to respective ambient O₃ condition. At Kranzberg Forest, the stomata-closing effect in elevated O₃ was consistently detectable, also through sap flow assessments at the level of individual sun and shade branches, and at the whole-tree foliage level (Matyssek et al., 2014a). The fact that enhanced O₃ levels caused differential stomatal behavior in the absence of effects on LMA (see above) supports the conclusion that leaf-anatomical changes (if any) may have been of secondary importance.

### 3.2. Seasonal course of stomatal O₃ response in both experiments

O₃-related reduction in stomatal conductance occurred in both experiments consistently in the absence of any decline in carboxylation capacity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) during early summer, i.e., June (Warren et al., 2007; Kitao et al., 2009; Hoshika et al., 2013b; Watanabe et al., 2014). Notwithstanding, the photosynthetic net CO₂ uptake rate temporarily showed decrease to some extent (Fig. 3, Kitao et al., 2009). Reduced CO₂ concentration in the leaf-intercellular space (Cᵢ) was observed during early summer under elevated O₃ (Fig. 4). Stomatal narrowing has been often reported as a secondary response to O₃ stress upon increase in Cᵢ as triggered by initial O₃-induced inhibition of carbon assimilation by chloroplasts (Reich, 1987; Weber et al., 1993; Farage and Long, 1995; Heath and Taylor, 1997). Stomata are known to be generally regulated toward sustaining the ratio of Cᵢ versus ambient CO₂ concentration (Cᵢ/C, Lambers et al., 2008). Such a pathway appears to be consistent with the finding from Siebold’s beech in late summer (Hoshika et al., 2013b; see Figs. 2–4; also see below). Observed stomatal
narrowing during early summer (June) is thus considered as a primary response to chronic O₃ impact. How to judge about the influence of enhanced O₃ impact on natural aging in deciduous leaves? Naturally aging leaves may display sluggishness in stomatal closure towards fall, even already before the onset of autumal discoloration, resulting in increasing Cᵢ (Schulze and Hall, 1982). Given the observed persistence or even decline of Cᵢ in late summer (August), the tendency of O₃ in reducing stomatal conductance apparently overrules seasonal trends of natural leaf aging. Such a conclusion does not conflict with mechanisms unveiled by molecular analysis, that demonstrate high capacity of O₃ for rapidly reducing stomatal width upon direct action of O₃-induced reactive oxygen species (ROS) and on abscisic acid (ABA) synthesis (Kangasjarvi et al., 2005; also see below).

Reduced Cᵢ in beech under enhanced O₃ regimes was consistent with an increase of the stable isotope ratios of ¹³C/¹²C. According to Farquhar et al. (1982), stomatal narrowing by impeding CO₂ influx leads to lowered discrimination of ¹³CO₂ relative to ¹²CO₂ during

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**Fig. 3.** Light-saturated net photosynthetic rate (A_{sat}) as a function of leaf mass per area (LMA) in leaves of European beech (upper figures) and Siebold’s beech (bottom figures) exposed to ambient (open diamonds, black line) or elevated O₃ (closed diamonds, dotted line). Each point is a single measurement. Measurements were conducted under 1500 μmol m⁻² s⁻¹ of PPFD, near ambient CO₂ concentrations (360 and 380 μmol mol⁻¹ in Kranzberg Forest and Sapporo Experimental Forest, respectively) and leaf temperature of 25 °C. The data were obtained in 2006 at Kranzberg forest (June: n = 15 in ambient and n = 15 in elevated O₃; August: n = 18 in ambient and n = 18 in elevated O₃), and in 2012 at Sapporo experimental forest (June: n = 21 in ambient and n = 21 in elevated O₃; August: n = 20 in ambient and n = 21 in elevated O₃; October: n = 20 in ambient and n = 21 in elevated O₃). p-value calculated from the ANCOVA with log-transformed LMA as covariate. n.s. denotes not significant (p > 0.05) (Modified after Kitao et al., 2009; Watanabe et al., 2014).

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**Fig. 4.** The ratio of intercellular to ambient CO₂ concentration (Cᵢ/Cₐ ratio) as a function of leaf mass per area (LMA) in leaves of European beech (upper graphs) and Siebold’s beech (bottom graphs) exposed to ambient (open diamonds, black line) or elevated O₃ (closed diamonds, dotted line). Each point is a single measurement. Measurements at both sites were conducted under 1500 μmol m⁻² s⁻¹ of PPFD, unchanged Cₐ, and leaf temperature of 25 °C. The data were obtained in 2006 at Kranzberg forest (June: n = 15 in ambient and n = 15 in elevated O₃; August: n = 18 in ambient and n = 18 in elevated O₃), and in 2012 at Sapporo experimental forest (June: n = 21 in ambient and n = 21 in elevated O₃; August: n = 20 in ambient and n = 21 in elevated O₃; October: n = 20 in ambient and n = 21 in elevated O₃). p levels of statistical significance were calculated through ANCOVA with log-transformed LMA as covariate. n.s. denotes not significant (p > 0.05). Since the slope of linear regression was not significantly different from zero in the response of Cᵢ/Cₐ ratio to LMA, potential effect of O₃ treatment was examined by t-test except for data from Siebold’s beech in June (modified after Kitao et al. (2009); Watanabe et al. (2014).
the fixation process by Rubisco, being reflected by increasing δ¹³C under enhanced O₃ impact. Such an outcome can plausibly be mediated through stomatal narrowing even at unchanged carboxylation capacity of Rubisco but concurrently minor effects on the net CO₂ uptake rate (cf. Löw et al., 2006; Kitao et al., 2009). Regarding the δ¹⁸O/δ¹⁶O isotope ratio in the leaf biomass, response to O₃ stress stayed unclear, as stomatal narrowing would have been expected to raise that ratio (Grams et al., 2007; Kitao et al., 2009). The leaf-level isotopic signatures, representing a seasonal integral of gas exchange performance, indicate long-term O₃ impact on leaf functioning, at least in the case of δ¹³C as observed at the German sites. An “optimal stomatal conductance model” developed by Hoshika et al. (2013b), which involves water, CO₂ and O₃ flux, suggests O₃-induced stomatal narrowing during early summer to reduce O₃ influx as counteracting O₃ injury (stress avoidance), allowing maximum photosynthetic capacity to be reached. Previous studies demonstrated O₃ to induce stomatal narrowing upon direct modulation of K⁺ channels (Torsethaugen et al., 1999; Vahisalu et al., 2010), alteration in Ca²⁺ homeostasis of guard cells (McAinsh et al., 2002) and phytohormonal production (Overmyer et al., 2008). The disturbance of ion fluxes by O₃ is related to down-regulated gene expressions such as those encoding Ca²⁺/H⁺ vacuolar antiporters (Dumont et al., 2014). Ablors et al. (2004) demonstrated the involvement of ABA signaling in O₃-induced stomatal closure using wild type and ABA insensitive mutants of Arabidopsis. Also in the Kranzberg beech trees, stomatal narrowing was related to induction of the ABA biosynthesis gene NCED1, whose transcripts were more abundant under enhanced O₃ treatment (Jehnes et al., 2007; Matyssek et al., 2010b). At the whole-canopy level, Matyssek et al. (2014a) show stomatal narrowing to reduce the seasonal O₃ influx by about 20% in proportion to the increase in O₃ exposure as caused by the 2xO₃ regime. It needs to stay open, though, of whether the stomatal response is an expression of O₃-driven regulation or incipient injury. Here the measurements of stomatal conductance were made under favorable conditions for stomatal opening, i.e., no midday depression in humid year. Given the O₃-incited ABA formation, reduction in stomatal conductance under O₃ stress may be the result of induced closure. Nevertheless, ABA once accumulated may impede opening as some kind of O₃ after-effect, reflected also by reduced stomatal conductance. Apparently, this issue cannot be decided definitively at the current stage. Similar findings of reduced stomatal conductance under elevated O₃ during the early growing season were also reported in European birch (Betula pendula: Oksanen, 2003) and plantain (Plantago major: Reiling and Davison, 1995). In contrast, several studies found that stomatal conductance did not decrease under elevated O₃ during early summer (Hanson et al., 1994; Grams et al., 1999; Watanabe et al., 2005). There are inter- and intra-species genotype variations of the effect of O₃ on stomata and on leaf metabolism (e.g., Furukawa et al., 1990; Pääkkönen et al., 1997). Such variations reflect different characteristics of stress resistance such as avoidance by stomatal narrowing and tolerance in terms of repair and detoxification capacities (e.g., Oksanen et al., 2007; Matyssek et al., 2008). O₃-induced stomatal narrowing, however, was diminished, and no difference in stomatal conductance between O₃ treatments was found during autumn in Japan (Fig. 2) in parallel to decreased photosynthetic capacity (Fig. 3), being in such a case mediated, perhaps, through increase in Ci (Fig. 4). Absence of O₃ effects on stomatal conductance during the late growing season (autumn) were reported similarly from Kranzberg Forest in 2004 (cf. Löw et al., 2007; Gessler et al., 2009), and other tree species (Betula pendula: Oksanen, 2003; Betula platyphylla var. japonica: Hoshika et al., 2013a; Quercus mongolica var. crispula: Watanabe et al., 2013) as well as from crops (Phaseolus vulgaris: Heagle et al., 2002). Interaction of O₃ impact and leaf age may be indicated in stomatal response. In late summer or autumn, the efficiency of O₃ stress avoidance through stomatal narrowing appears to be diminished (Hoshika et al., 2013b). This is in line with previous studies suggesting enhanced chronic O₃ exposure to mitigate stomatal narrowing associated with sluggishness and imprecision in regulation (Paoletti, 2005; Paoletti and Gruhke, 2005; Mills et al., 2009; Hoshika et al., 2012a). Mechanism underlying stomatal sluggishness under O₃ stress, however, still await clarification. Limited lignification of guard and subsidiary cells may be one cause under O₃ exposure (e.g. in Picea abies, Maier-Maercker, 1989), whereas no such limitation existed in O₃-injured leaves of manna ash (Fraxinus ornus) while showing stomatal sluggishness (Paoletti et al., 2009). Omasa (1990) reported that such stomatal response was caused by slight increase in permeability of epidermal cell membranes and alteration of the osmotic pressure modulating a balance in turgor between the guard and subsidiary cells. Mills et al. (2009) found O₃ to reduce stomatal sensitivity to ABA, which may be mediated through O₃-induced ethylene emissions (Wilkinson and Davies, 2010). Moreover, gene expression was recently demonstrated under O₃ impact to inhibit CO₂ signaling involved in stomatal closure (Dumont et al., 2014). According to Paoletti et al. (2009) stomatal sluggishness may be indicative of accelerated senescence in leaf metabolism. In general, stomata of deciduous leaves tend to lose effective responsiveness to environmental stimuli at the end of the growing season, being called “dull leaf” phenomenon (Terashima, 2002; cf. Schulze and Hall, 1982). Apparently, the impact of O₃ on stomatal regulation during leaf aging is complex.

4. Conclusion

Our findings provide evidence that O₃-induced stomatal narrowing occurred in trees of European and Siebold’s beech that expand in dimension. Reduction of stomatal conductance coincided with unchanged carboxylation capacity and mesophyll conductance during early summer, which did not rule out, however, temporary restriction of the photosynthetic net CO₂ uptake rate to some extent. Observed O₃-induced stomatal narrowing was, however, diminished in autumn in both sites. This emphasizes interaction in stomatal regulation between O₃ impact and seasonal leaf senescence.

Recent O₃ risk assessments for forest trees are focused on a stomatal O₃ flux basis (Matyssek et al., 2007a; Karlsson et al., 2007; Sitch et al., 2007; Mills et al., 2010). Limitation of stomatal conductance under elevated O₃ occurred to similar extents in both experiments. Such O₃-induced stomatal narrowing may lead to further limitation of O₃ influx to a leaf, although it cannot be decided as to whether such an outcome results from incipient O₃ injury rather than stomatal acclimation to O₃ stress. Current O₃ risk modeling for terrestrial ecosystems do incorporate O₃-induced stomatal narrowing, presuming monotonic decrease of stomatal conductance with cumulative O₃ exposure or cumulative O₃ flux to be accompanied by O₃-induced decline of photosynthesis (e.g., Felzer et al., 2004; Sitch et al., 2007; Collins et al., 2010). However, the response of O₃-induced stomatal narrowing as observed in beech under the two free-air O₃ exposure experiments was more complex than currently covered by modeling. Stomatal narrowing was initiated already during early summer in beech at both sites, and such response was diminished by autumn. The presented account consolidates knowledge on stomatal O₃ response in view of strengthening modeling concepts and their validation related to O₃ risk assessment in forest trees.
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References


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