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Review

Advances in understanding ozone impact on forest trees: Messages from novel phytotron and free-air fumigation studies

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Novel phytotron and free-air O_3 exposure studies on forest trees communicate sensitivity to be governed by genotype, ontogeny and biotic agents rather than species per se.

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ABSTRACT

Recent evidence from novel phytotron and free-air ozone (O_3) fumigation experiments in Europe and America on forest tree species is highlighted in relation to previous chamber studies. Differences in O_3 sensitivity between pioneer and climax species are examined and viewed for trees growing at the harsh alpine timberline ecotone. As O_3 apparently counteracts positive effects of elevated CO_2 and mitigates productivity increases, response is governed by genotype, competitors, and ontogeny rather than species *per se*. Complexity in O_3 responsiveness increased under the influence of pathogens and herbivores. The new evidence does not conflict in principle with previous findings that, however, pointed to a low ecological significance. This new knowledge on trees' O_3 responsiveness beyond the juvenile stage in plantations and forests nevertheless implies limited predictability due to complexity in biotic and abiotic interactions. Unravelling underlying mechanisms is mandatory for assessing O_3 risks as an important component of climate change scenarios.

1. Introduction

Ground-level ozone (O_3) concentrations have become a factor of growing concern that may impose high risk of injury and productive decline on vegetation relative to other air pollutants (Lefohn, 1992; Matyssek and Sandermann, 2003; Sitch et al., 2007; Fowler et al., 2008; Wittig et al., 2007, 2009). Awareness has changed from regarding O_3 risk as a local threat towards a regional and global phenomenon (Keating et al., 2004), recognizing intercontinental drifts of air masses in the troposphere and associated formation and transport of enhanced O_3 plumes (Fabian, 2002; Li et al., 2002; Newell and Evans, 2000). In view of such evidence, ground-level O_3 has encountered less attention relative to other factors of "Global Change" (like air temperature, drought, atmospheric CO_2 or nitrogen deposition) and their effects on vegetation (IPCC, 2007). This is remarkable, as O_3 is known to have the capacity of counteracting stimulation of photosynthesis and biomass production by elevated CO_2 (e.g. Karnosky et al., 2003a, 2007a,b; Grams et al., 1999, 2007; Wittig et al., 2009) and affecting carbon flux at the global scale. Risk may even become exacerbated, as further increase in ground-level background O_3 concentrations has been predicted across parts of the northern hemisphere, along with new "hot spots" arising in Asia, Central Africa and South America (Fowler et al., 1999, 2008; Vingarzan, 2004; Dentener et al., 2006). Given such predictions, modelling indicates substantial decline in the forest-dominated land carbon storage upon O_3 impact by the end of this century, which in this way contributes to the radiative forcing of the atmosphere with its increasing CO_2 level (Sitch et al., 2007).

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

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In relation to published prognoses and modelling of forest performance under O_3 impact, empirical databases and mechanistic understanding of tree and stand responses, in particular, of relevance for site conditions, are scarce (Matyssek et al., 2008). Such kind of knowledge represents a pre-requisite, however, for reliable model development and validation. Instead, the literature has been dominated throughout decades by the evidence gathered from the controlled conditions of chamber studies, which – although being valuable for unveiling response mechanisms – possess limited ecological relevance for natural site conditions (Musselman and Hale, 1997). This limitation has been characterized by micro-climatic bias and the use of early ontogenetic stages of trees, grown in the absence of most abiotic (except for O_3) and biotic constraints (e.g. competition, parasites; Kolb and Matyssek, 2001) – such settings being altogether conducive to O_3 injury. Methodological advances have recently become available, however, which strengthen ecological relevance of experimental results – (1) either through novel experimentation in phytotrons (Grams et al., 2002), or (2) free-air O_3 fumigation technologies (Karnosky et al., 2001, 2007b; Werner and Fabian, 2002).

Regarding (1), effects of intra and inter-specific competition were analyzed, for the first time, on the sensitivity of juvenile climax tree species to combined O_3/CO_2 regimes, including an O_3/pathogen interaction, although the influence of O_3 on competition had been addressed before in a chamber study on an early-successional plant community (Barbo et al., 1998, 2002). In the recent phytotron experiment, trees were studied in mixed or monoculture up to an age of six years, using O_3 and climate regimes previously recorded at a forested site. The phytotron system employed was unique in approaching the natural light spectrum and diurnal courses, and in decoupling below from aboveground thermal control (Payer et al., 1993). Although it is – intrinsically – not the purpose of even advanced phytotron systems to replace outdoor studies, experimental designs can approximate field conditions and make factorial associations encountered at field sites accessible for analysis. Examples are biotic impacts modifying the plant's O_3 response, as respective evidence had been lacking for woody-plant systems (Matyssek and Innes, 1999; Kolb and Matyssek, 2001). Concerning (2), free-air O_3 fumigation studies on field-grown trees have been conducted for several years at three locations in Europe and the USA (Karnosky et al., 2001, 2007b; Matyssek et al., 2007a; Oksanen et al., 2007). Focus was on adult or maturing trees of climax or pioneer species grown in forest stands/plantations and exposed to experimentally enhanced O_3 or combined O_3/CO_2 regimes, including competitive interactions and parasitic impacts. Free-air fumigation can be unrestrained by micro-climatic bias while allowing fumigation to tall forest trees having advanced ontogeny, thus providing a high degree of ecological relevance. Nevertheless, control treatments are in fact restricted – as opposed to chamber studies – to the site-specific ambient O_3/CO_2 regimes rather than treatments with below-ambient gas concentrations. In such respects, chamber and free-air studies may be viewed as complementary approaches, with the new methodology representing a tool for building upon previous chamber studies and adding greater capacity to extrapolate results. This review will highlight and compare the main findings from the two approaches addressed above, based upon (1) the phytotron approach reported by Kozovits et al. (2005a,b), Luedemann et al. (2005, 2009) and Grams et al. (2002, 2007) and (2) the free-air O_3 fumigation experiments conducted in Freising/Germany (Kranzberg Forest, e.g. Matyssek et al., 2007a), Rhinelander/USA (Aspen FACE, e.g. Karnosky et al., 2007b) and Kuopio/Finland (e.g. Oksanen et al., 2007). For the first time, key messages from these studies will be cross-compared from a unified perspective, relating findings to current knowledge and highlighting the state of science conducted previously to the experiments summarized here. In doing so, the focus will be on the genera *Betula, Populus, Acer, Fagus* and *Picea*, while exploring the extent to which differential O_3 sensitivity is reflected between pioneer *versus* climax tree species (cf. Harkov and Brennan, 1982; Reich, 1987). While considering genotypic variation, outcomes will be examined for their potential validity under harsh environmental conditions, using datasets provided by Havranek et al. (1989), Volgger (1995), and Wieser et al. (2001) on the O_3 sensitivity of pioneer and climax tree species growing at the timberline ecotone of the Central-European Alps (i.e. *Larix decidua* Mill. *versus Pinus cembra* L. and *Picea abies* (L.) Karst, respectively). Fig. 1 depicts the rationale and structure for this review, illustrating the sequence of approaches presented and interrelating the accumulated evidence.

2. O_3 sensitivity of pioneer trees

2.1. Knowledge on birch and poplar prior to free-air O_3 fumigation studies

Juvenile birch and poplar have been the most intensively studied pioneer trees regarding impact and effects of enhanced chronic O_3 regimes under controlled chamber conditions (Kolb and Matyssek, 2001; Matyssek, 2001; Matyssek et al., 1998; Pääkkönen et al., 1995, 1998; Karnosky et al., 2007a). At 2–3-times higher O_3 exposure of *Betula pendula* Roth. and *Populus tremula* L. than prevailing in the ambient air (e.g. near Zurich/Switzerland), a pronounced decrease in photosynthesis was typically found, being mediated through collapse of leaf mesophyll cells (Matyssek et al., 1991, 1993a; Günthardt-Goerg et al., 1997). Decline was also accompanied by conspicuous, O_3-induced leaf discoloration (Günthardt-Goerg et al., 1993, 1996, including *P. tremula*; Matyssek et al., 1990, 1993a, 1998). Progress in O_3-driven leaf injury depended on nutrition and the time of flushing during the season, due to the differently advanced ontogenies of each leaf in these tree species that have indeterminate, seasonal shoot elongation growth associated with continued leaf formation (Landolt et al., 1997; Maurer et al., 1997; cf. Bagard et al., 2008). During decline, the balance between leaf photosynthesis and transpiration mostly turned towards decreased water-use efficiency (WUE), while stomatal density and conductance could stay unchanged, be increased or decreased (Frey et al., 1996; Matyssek et al., 1998; Matyssek and Sandermann, 2003). Declining WUE did not necessarily result,

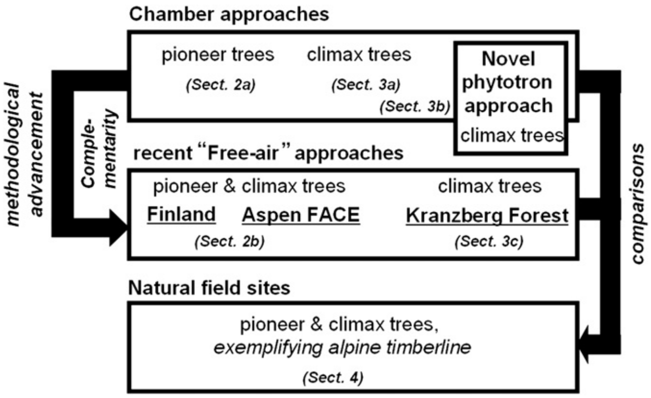

Fig. 1. Overview on the rationale and structure of the review, as defined by the account of the outcome from major methodological approaches, with emphasis on recent phytotron and free-air O_3 fumigation studies. Arrows denote methodological advancement and complementarity of approaches (left) and the direction of outcome comparisons (right). Sect. 2a in the figure corresponds to sect. 2.1 in the text, 2b to 2.2, 3a to 3.1, 3b to 3.2 and 3c to 3.3.

however, in decreased $\delta^{13}C$ of plant biomass (cf. Farquhar et al., 1989), rather an increase was observed (Matyssek et al., 1992; Saurer et al., 1995). This effect was related to stimulated PEPc relative to reduced Rubisco activity in CO_2 fixation (cf. Fontaine et al., 1999), due to the low discrimination of $^{13}CO_2$ by PEPc relative to Rubisco (Saurer et al., 1995). PEPc is a common enzyme in C_3 plants, feeding the citric cycle through non-photosynthetic CO_2 fixation (as an anaplerotic pathway) under high C demand (Wiskich and Dry, 1985), as potentially represented by defence of O_3 stress. Saurer et al. (1995) found linearity between $\delta^{13}C$ and external O_3 exposure indicating a constant ratio of the exposure to the physiologically relevant O_3 flux into the leaves, given the high rate of air mixing in chambers. PEPc apparently supported O_3 detoxification and repair of injury (Dizengremel, 2001), accompanied by favoured glycolysis (Landolt et al., 1997; Einig et al., 1997; cf. Dizengremel et al., 1994). The metabolic status resembled end-product inhibition of photosynthesis, exacerbating carbon limitation, and was mediated in leaves through disrupted assimilate translocation upon cell collapse. Additional starch accumulation along leaf veins reflected disturbed phloem loading (Matyssek et al., 1992; Günthardt-Goerg et al., 1993).

Given the increased assimilate demand in leaves for detoxification and repair *versus* inhibited translocation, other tree organs became carbon-limited under O_3 stress. Often, the root system was affected most, and in stems radial rather than longitudinal growth was limited (Matyssek et al., 1992, 1993a,b, including *Populus* × *euramericana*). As stems tended to develop similar numbers of nodes irrespective of O_3 stress, changes in biomass partitioning did not appear to merely reflect delayed ontogeny (Matyssek et al., 1998; cf. Walters et al., 1993). Nonetheless, lateral branching became suppressed – an effect that limited whole-plant biomass production substantially in addition to photosynthetic impairment, changed leaf morphology and premature leaf loss (Matyssek et al., 1992; Matyssek, 2001). Similar to leaf level responses, the high rates of air mixing in chambers favoured linearity between O_3 exposure, declining whole-plant biomass production and altered carbon allocation. Matyssek and Sandermann (2003) introduced a scaling scheme, linking O_3 responses across the cell, organ and whole-plant level. Nevertheless, other interacting factors, e.g. nutrition, can distinctly modify scaling patterns, with the leaf life span achieved under O_3 stress becoming a determinant of whole-tree performance (Maurer and Matyssek, 1997; Matyssek, 2001).

These principles of whole-tree response were corroborated in Finland by three short-term O_3 fumigation studies (less than one growing season each: Pääkkönen et al., 1995, 1998; Oksanen and Holopainen, 2001) and one throughout three consecutive growing seasons (Riikonen et al., 2004), conducted on juvenile *B. pendula* in laboratory or open-top chambers (OTC) in the field (Appendix, Table 1). These short-term studies yielded O_3-induced declines in foliage area by 10–17%, as concurrent dry mass increments of stems and root systems were reduced by 1–8%, and by 10%, respectively. Stem height growth was reduced by 5–7%. Cumulative reductions became larger during the three-year study. Also in these four chamber studies, stem biomass development was negatively related to O_3 exposure (still expressed in Fig. 2 as AOT40 at that time rather than O_3 flux). These growth reductions were accompanied by visible foliar symptoms, ultra-structural injuries especially in chloroplasts, reduced net photosynthesis and impaired stomatal regulation. High O_3 sensitivity was reported also from other pioneer tree species under controlled conditions, such as *Populus tremuloides* or *Prunus serotina* (cf. Kolb and Matyssek, 2001; Matyssek and Sandermann, 2003).

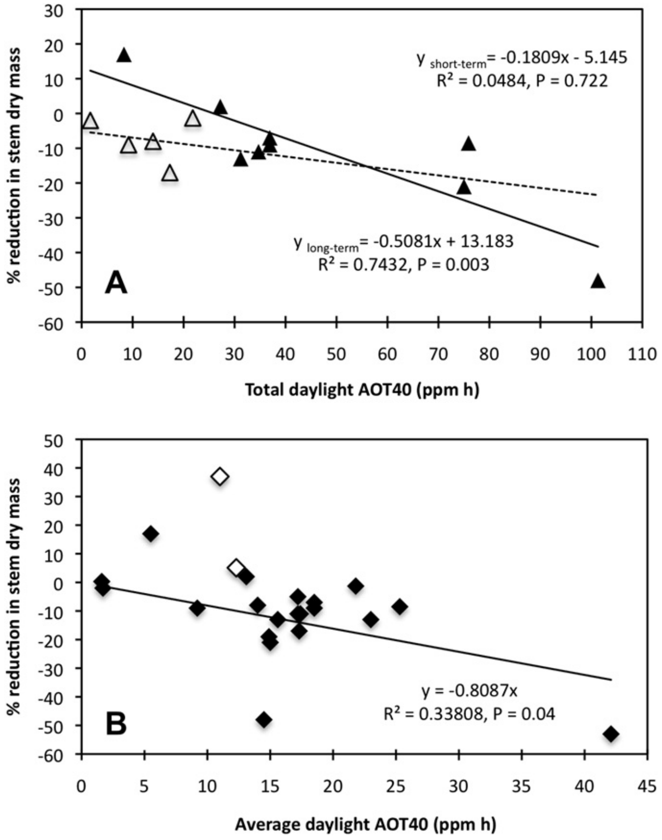

Fig 2. A. The relationship between percentage (%) reduction on stem dry mass accumulation of birch species (*Betula pendula* and *B. pubescens*) and total O_3 exposure (daylight AOT40 exposure over the whole experimental period). The relationships were analyzed as linear regressions. Symbols: (▲) long-term experiments (≥2 growing seasons), conducted with free-air fumigation system; (△) short-term experiments (≤1 growing season), conducted mainly in growth chambers or open-top chambers. Each datapoint represents aggregated data for all genotypes at the end of each experiment. The continuous line represents the linear regression for the long-term experiments and the dashed line represents the short-term experiments. The references for short-term and long-term experiments are given in Appendix Table 1. B. The relationship between percentage (%) reduction on stem dry mass accumulation of Finnish pioneer species (*Betula* sp. and *Populus* sp.), conifers (*Picea abies*, *Pinus sylvestris*) and daylight AOT40 exposure (average per growing season). Symbols: (▲) pioneers; (◇) conifers. Each datapoint represents aggregated data for all genotypes at the end of each experiment. The trendline represents the linear regression for the pioneers only (for conifers a trendline could not be drawn due to low number of datapoints). The references for pioneer species and conifers are given in Appendix Table 1.

Karnosky et al. (2007a) have provided a comprehensive review on O_3 exposure studies with forest trees including the evolution of experimental technologies spurred by early field observations in the late 1950s. These early correlative associations between high levels of photochemical oxidants and foliar injury led to some 45 years of laboratory, greenhouse, OTC, and free-air experiments. Collection of aspen clones from across the natural range in the United States by Berrang et al. (1989) led to a series of chamber studies by D.F. Karnosky and co-workers on the sensitivity of aspen genotypes to both O_3 and CO_2 separately or in combination. These experiments characterized the physiological mechanisms for O_3 responses from which exposure-response relationships were developed (Coleman et al., 1995a,b; Karnosky et al., 1996, 1998; Kull et al., 1996; Kubiske et al., 1998; Isebrands et al., 2000). Most of these studies are in agreement that aspen grown in OTC can be stimulated by up to 28% under elevated CO_2 concentrations if other factors such as soil nutrient status, water availability, temperature, and pest activity were held constant (Isebrands et al., 2001). Increases in productivity were likely due to increases in whole-crown leaf area, leaf duration, and leaf area index (LAI) (Ceulemans and Mousseau, 1994; Curtis et al., 1995), owing to greater photosynthetic rates and whole-plant photosynthesis (Kubiske et al., 1997).

All carbon flows into trees through leaves via the process of photosynthesis. However, there is considerable variability in photosynthetic O_3 response between aspen genotypes (Coleman et al., 1995b). Accelerated leaf aging due to O_3 exposure is a well-documented phenomenon in aspen (Karnosky et al., 1996). Ultimately, in the case of the genus *Populus*, there is a very wide range of O_3 sensitivity at the genotype level. Growth of some clones actually appeared to be slightly stimulated under elevated O_3 regardless of CO_2 treatment (Karnosky et al., 2007a). Open-top chamber studies with aspen and hybrid poplar have shown that height growth was often not affected, while diameter growth decreased significantly (Karnosky et al., 1996; Dickson et al., 1998). Ozone often decreases carbon allocation to root systems (Andersen, 2003). In aspen, O_3 also seems to alter ^{14}C distribution to roots (Coleman et al., 1995a), and differentially impacts root growth (Karnosky et al., 1996). Carbon allocation and partitioning to roots can in fact be dramatically altered by O_3 (Coleman et al., 1995a) leading to decreased root growth of aspen (Coleman et al., 1996) and altered root/shoot ratios (Karnosky et al., 1996). Experiments conducted in the US have reported a range of O_3 responses in photosynthesis, stem height/diameter relationships and below *versus* aboveground carbon partitioning that is similar to that reported in Europe.

In hybrid poplar clones, Dickson et al. (1998) reported that the effect of elevated CO_2 on negating the detrimental effects of elevated O_3 varied by clone. Kull et al. (1996) had initially reported that elevated CO_2 ($+150\ \mu l\ l^{-1}$) increased O_3 susceptibility of two aspen clones as measured by photosynthetic responses. Further work by Karnosky et al. (1998) in open-top chambers in natural soil then showed that elevated CO_2 did not in fact make O_3 effects worse, as had been predicted. However, CO_2 also did not ameliorate adverse effects of O_3 exposure over a three-year long fumigation.

2.2. Pioneer in relation to climax tree species under free-air O_3 fumigation

2.2.1. Evidence from the United States

A common finding from the extensive suite of O_3 exposure studies conducted in chamber experiments is the high O_3 sensitivity of seedling to sapling stage trees when grown with adequate water and nutrient supply (Karnosky et al., 2007a). In most cases, these trees were also exposed in the absence of competition or other major biotic agents (i.e. pests, mycorrhizae). Another common aspect of the experimental designs was that the seedling or juvenile trees often belonged to relatively fast-growing pioneer species (Kolb and Matyssek, 2001). Although very useful in demonstrating physiological mechanisms, and proving that (in the case of OTC) background ambient O_3 concentrations could elicit symptoms and reduce growth, trees grown in chambers were sometimes different than seedlings of the same source and age growing outside at the same site (Karnosky et al., 2007a). Eventually, using a common set of aspen clones, Karnosky et al. (2006) provided evidence for at least some level of commonality in O_3 response between OTC, FACE, and ambient studies in the same region. From a risk analysis perspective then, the question arises: Can direction and magnitude of O_3 effects reported in chamber studies be carried over to trees grown in field plantations? In particular, when inter-annual climate variation (changes in temperature and precipitation patterns), species life history, stand dynamics, and pests are allowed to interact naturally during multi-year O_3 fumigation, is there a commonality in response between chamber and field exposure situations?

In response to the above questions, it is necessary to allow for the natural operation of boundary layers in the pathway of O_3 uptake, remove most of the bias of micro-climatic conditions and, hence, normalize physiological responsiveness (Karnosky et al., 2001). Therefore, the potential use of open-air (FACE) systems for delivering reliable concentrations of O_3 to maturing trees in large plot sizes (stands) was proposed by Hendry et al. (1999). One such example of the open-air concept originally proven by McLeod (1995), is the Aspen Free-Air Carbon Dioxide Enrichment (FACE) experiment located on a 32 ha site in northern Wisconsin, USA. At Aspen FACE, aggrading stands of two intolerant pioneer species [trembling aspen (*P. tremuloides* Michx.); paper birch (*Betula papyrifera* Marsh.)], and one tolerant climax hardwood species [sugar maple (*Acer saccharum* Marsh.)] have been exposed to O_3 from the seedling to past-canopy closure (for aspen plantations) to harvest over a twelve-year period (1998–2009). The Aspen FACE experiment consists of a full factorial design comprising twelve 30 m diameter rings, including three control (ambient) and three elevated O_3 rings. The eastern half of each ring was planted in two-tree plots (1 m × 1 m spacing) with 5 clones having different phenology, and selected from previous studies (Karnosky et al., 1996) to cover a variety of responses to O_3 exposure. Responses ranged from positive (slight growth stimulation), through various degrees of tolerance, to extreme sensitivity (Percy et al., 2007, in press). The north-western quadrant was planted (1 m × 1 m) with alternating aspen clone 216 and sugar maple; the south-western quadrant was planted (1 m × 1 m) with alternating aspen clone 216 and paper birch.

The rings were planted in late 1997. Treatments were applied from bud break to bud set from 1998 until 2009. Details on fumigation technology, system performance and exposure levels are provided in Karnosky et al. (2003a,b, 2005, 2007b). Effects of elevated concentrations of O_3 and/or CO_2 on gas exchange showed considerable intra-specific variability. Early-successional aspen and birch have been generally responsive, while the late-successional sugar maple has not been nearly as responsive, at least to date (Karnosky et al., 2005). Effects of O_3 on aspen photosynthesis, growth, and biomass have been negative, while those of elevated CO_2 have been positive. Co-exposure of both gases has demonstrated that O_3 at elevated concentrations (ca. 1.3–1.5 × ambient) has the potential to offset the positive effects of elevated CO_2 (Percy et al., 2002; Karnosky et al., 2005; King et al., 2005). Leaf area index in aspen was reduced under O_3 due to a combination of delayed bud break, and accelerated leaf senescence/abscission with consequences to carbon gain (Karnosky et al., 2005). Birch, when grown with aspen has shown a relatively high degree of tolerance for O_3. After 6 years of exposure, birch biomass increased by 45% under elevated CO_2 and decreased by 13% under elevated O_3. In contrast, aspen biomass increased by only 25% under CO_2 and decreased more than birch (by 23%). When exposed to both gases in combination, the competitive advantage gained by birch tolerance to O_3 was evident; aspen biomass was reduced by 8% relative to control. Conversely, birch biomass increased by 8% (King et al., 2005).

Most conspicuous was the finding that paper birch did not show any O_3 response over the first eight years of fumigation in height and diameter growth under free-air O_3 fumigation (Kubiske et al., 2006). This finding is in marked contrast with observations from previous controlled chamber studies on this species (see sect. 2.1). However, paper birch did exhibit a growth response at Aspen FACE when exposed concurrently to elevated CO_2 (Kubiske et al., 2006). Still, the yield in stem height and volume was higher than in the control (ambient CO_2) irrespective of O_3 treatment, as elevated CO_2 had a strong stimulating effect on stem growth. Trembling aspen, the other pioneer tree species of Aspen FACE, resembled paper birch under the same experimental conditions in that elevated CO_2

also stimulated stem height and volume increment (Kubiske et al., 2006). However, as opposed to paper birch, elevated O_3 reduced stem growth in trembling aspen, and, thus, O_3 exposure offsets the positive growth effect from elevated CO_2. In other words, under elevated $CO_2 + O_3$, aspen growth was not significantly different from the control treatment (Kubiske et al., 2006).

Growth changes reported following aspen or birch exposure to elevated CO_2, O_3, or $CO_2 + O_3$ represent hierarchical, "bottom-up" mediated changes from alterations in cellular, leaf-level biochemical, and physiological parameters (Karnosky et al., 2003a,b). After five years of O_3 exposure, paper birch manifested enhancements in peroxisomal activity, stomatal conductance during drought, along with increased susceptibility to stem dieback and herbivory activity (Oksanen et al., 2003). Prior to year five of fumigation, annual stem increment had not been affected by O_3 (see above). Trembling aspen was consistent with paper birch in displaying enhancements in peroxisomal activity, stomatal conductance and susceptibility to pests. In addition, aspen showed a reduction in photosynthesis, leaf life span, and LAI under O_3 stress (Karnosky et al., 2005).

Of particular interest at Aspen FACE has been the response of the late-successional, climax species sugar maple. No statistically significant effects of O_3 were detected three years after fumigation began. Consistently, sugar maple stem growth was hardly affected by elevated O_3 and/or CO_2 regimes throughout the first 8-year period, as the annual stem production in absolute terms was substantially lower in the climax than pioneer species, irrespective of the exposure regimes (Kubiske, 2007). Previously, at high O_3 concentrations (150 or 200 nl l^{-1}), juvenile (45-day-old) sugar maple seedlings exposed in chambers had a reduced assimilation rate and Rubisco content after almost two months, suggesting that enhanced reducing power and structural carbon production was required for detoxification and repair of oxidant damage (Gaucher et al., 2003). While sugar maple seedlings have been previously shown to respond (biomass reduced 39–49%) to O_3 over a three-year fumigation in OTC, the physiological mechanisms of response were complex and required scaling from the leaf level (understanding of source–sink relationship) in order to be predictable (Topa et al., 2001, 2004). These findings with sugar maple, as well as the onset of birch decline at Aspen FACE in some elevated O_3 rings following severe drought and insect attack, clearly demonstrate the requirement for long-term studies of interacting biotic/abiotic stresses under free-air-like field situations. Long-term, stand-level responses of two pioneer species (aspen, birch), and one climax species (sugar maple) cannot always be predicted from shorter-term, chamber studies.

Nevertheless, the response patterns just described cannot be generalized. Responsiveness was strongly governed by species life history, and strong genetic variability was observed within aspen (Karnosky et al., 2005). Of the five aspen genotypes examined at Aspen FACE, one clone displayed a strong reduction in stem volume under elevated O_3, but not under elevated CO_2 (Kubiske, 2007). Another clone performed in an opposite way – no response to O_3, but strong stimulation of stem growth under elevated CO_2. A third clone showed an inhibitory effect by O_3 and a stimulatory one by CO_2, resulting in stem performance under co-exposure to O_3 and CO_2 that was similar to that measured under ambient (control) conditions (Kubiske, 2007).

Most remarkable, apart from genetic influences, was the influence of competition in the aspen plantations on tree responsiveness to O_3 and/or CO_2 fumigation. In the case of trembling aspen, the "relative competitiveness" of this species, expressed as the proportional biomass accumulation in plantations throughout the 8-year period, varied greatly depending upon the competing tree species – either paper birch (the pioneer) or sugar maple (the climax species; Kubiske, 2007). In the presence of paper birch, trembling aspen became gradually suppressed under ambient (control) conditions, but not when grown in competition with sugar maple. Suppression of aspen by co-planted birch was also enhanced under elevated O_3 or CO_2. The "relative competitiveness" of trembling aspen did not differ under co-exposure to elevated O_3 and CO_2 from that under ambient O_3/CO_2 concentrations. All such effects occurring in trembling aspen when competing with paper birch were absent, however, when the competitor was sugar maple (Kubiske, 2007). In the latter case, trembling aspen dominated competition, irrespective of treatment. Given differential responses of tree species to elevated background and/or high stochastic pulses of tropospheric O_3, it is likely that plant–plant interactions will also be affected.

However, it is difficult to predict the important long-term effects of O_3 on successional processes in forest communities (Laurence and Andersen, 2003). Clearly the response of an individual to environmental stressors almost always differs from the taxon's response to the same stressors, in competition (Poorter and Navas, 2003; Liu et al., 2004; Kozovits et al., 2005a,b; Barbo et al., 2002). To demonstrate this, the "importance index" based upon number of surviving individuals and their stem volume as calculated by Kubiske (2007) is extremely instructive. For instance, in the aspen monoculture consisting of five clones, clone 259, which was the weakest competitor in the Aspen FACE experiment, exhibited the greatest increase in O_3-induced competition effects relative to its performance in the control treatments (Fig. 3). Following two more years of O_3 treatment, and after the publication of those data, clone 259 was entirely eliminated from the elevated O_3 treatments (Kubiske, unpublished data).

Under such conditions, it would be reasonable to expect the dominant competitor to realize further gains as O_3-sensitive taxa become suppressed. This assumption was validated in the common garden study under the ambient O_3 gradient aforementioned (Karnosky et al., 2003a). Differential O_3 effects can in fact

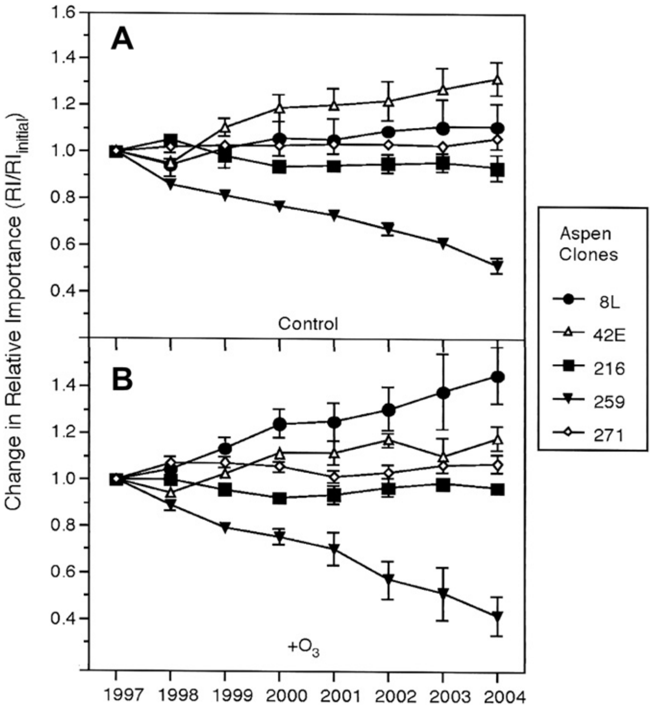

Fig. 3. Changes in relative importance (RI) of five, co-occurring trembling aspen clones exposed to A) background O_3 (control, 20–40 nl l^{-1}) and B) elevated O_3 ($+O_3$, 60–80 nl l^{-1}) in a free-air O_3 exposure experiment. Relative importance was calculated from clone relative volume plus clone relative numbers. Modified from Kubiske (2007).

restructure competitive assemblages in unpredictable ways. In the aforementioned FACE experiment, aspen clone 8 L was a moderate competitor in the control treatments, but it emerged as the dominant competitor in the aspen monoculture under elevated O_3 (Kubiske, 2007; Fig. 3). Likewise, decline of O_3-sensitive aspen clones, in competition with several tolerant clones in common garden plots, increased directly with exposure to ambient O_3 (Karnosky et al., 2003b). Similar patterns have been observed in naturally occurring forests of aspen, and eastern white pine (*Pinus strobus* L.), ponderosa pine (*P. ponderosa* Dougl. ex Laws) and Jeffrey pine (*P. jeffreyi* Grev. & Balf.) (Karnosky, 1981; McBride et al., 1985; Berrang et al., 1989; Patterson and Rundel, 1995).

One common consequential effect from longer-term exposure O_3 above certain levels is a tendency for predisposition of a host tree to increased insect attack and disease incidence. At Aspen FACE, elevated O_3 stimulated *de novo* synthesis of free fatty acids by up to 23%, and altered epicuticular wax chemical composition leading to a marked reduction in aspen leaf surface crystalline micro-structure (Percy et al., 2002; Mankovska et al., 2005). Notable was the increased abundance of fatty acids and hydrocarbons, chemical cues for sap-feeding insects (Percy et al., 2002). Such changes to the phylloplane have been shown to be associated with effects on leaf surface properties including increased wetting/rain retention, resulting in 2–5 fold increase in incidence and severity of the common foliar rust *Melampsora medusae* under elevated O_3 (Karnosky et al., 2002). Feeding by aphids (*Chaitophorus stevensis*) that infest trembling aspen throughout its North American range increased under elevated O_3 (Percy et al., 2002). This was likely a top-down mediated response (Mondor et al., 2004a), and not a consequential effect of changes to leaf surface properties.

Along with improved host recognition, plant synthesis of phenolic glycosides, which serve as defence against insect herbivores, decreased in elevated O_3 (Percy et al., 2002). Similarly, levels of defensive phytochemicals including phenolic glycosides and condensed tannins decreased in elevated O_3. This resulted in lengthened forest tent caterpillar (*Malacosoma disstria*) development times, increased dry mass consumption, and female pupal mass (Kopper and Lindroth, 2002). The size of female *M. disstria* is directly related to number of offspring, suggesting that increased O_3 could increase the severity and frequency of outbreaks of this important defoliator in North America (Percy et al., 2002). However, increased O_3 decreased the occurrence of the aspen blotch leaf miner (*Phyllonorycter tremuloidiella*) as the rate of oviposition decreased (Kopper and Lindroth, 2003).

Greater O_3 tolerance of paper birch compared to aspen, which affected community dynamics described above, might translate to less severe effects on trophic level interactions. Elevated O_3 had no effect on the synthesis of phenolic glycocides in paper birch, and thus no effect on the defoliating insect papermarked tussock moth (*Orgyia leucostigma*) (Kopper et al., 2001). Similarly, the relative growth rate, development time, adult weight and embryo number of a birch aphid (*Cepegillettea betulaefoliae*) were unaffected by elevated O_3 (Awmack et al., 2004). In addition to direct plant–insect interactions, pheromone-mediated escape responses of aphids (*C. stevensis*) as a defence against natural enemies improve in elevated O_3, rendering the aphids less susceptible to parasitism or predation (Mondor et al., 2004a). Other species of aphids responded to elevated O_3 by altering genotypic and phenotypic frequencies (Mondor et al., 2004b, 2005). In particular, the number of winged offspring increased in elevated O_3 in the presence of hymenopteran parasitoids (Mondor et al., 2004b).

Although the climax tree species at Aspen FACE tended to be relatively insensitive to the O_3 and CO_2 treatments, as has previously been reported by Harkov and Brennan (1982), Reich (1987), and Topa et al. (2001, 2004), generalization is difficult. Apparently, individual species life history, genotype, competition, and pest activity all have the capacity to dominate tree response to gas regimes. Increased tropospheric O_3 has previously been shown to alter competitive interactions among species (Arbaugh et al., 2003; Grams et al., 2002; Liu et al., 2004; Kozovits et al., 2005a,b). In the northeastern U.S., trembling aspen frequently co-occurs with paper birch, and is a common pioneer species replaced by later successional sugar maple on mesic sites. Results from the Aspen FACE experiment suggest that under elevated O_3 aspen is competitively disadvantaged compared to either paper birch or sugar maple (Kubiske, 2007). This suggests that if tropospheric O_3 pollution continues to rise, mixed aspen-birch stands may gradually become dominated by paper birch. Furthermore, the successional replacement of aspen by sugar maple could be accelerated.

2.2.2. *Evidence from Finland*

In Finnish pioneer species *Betula* sp. and *Populus* sp. average growth reductions in different free-air O_3 fumigation experiments (Table 1 and references therein; average AOT40 exposure 16 $\mu l\ l^{-1}$ h/growing season) have been 15% for foliage area, 3% for stem height growth, 12% for stem dry mass, 19% for root dry mass, and 14% for radial growth as compared to control plants (Fig. 2; Pääkkönen and Holopainen, 1995; Pääkkönen et al., 1996, 1997a,b; Oksanen and Saleem, 1999; Oksanen, 2001, 2003a,b; Saleem et al., 2001; Kontunen-Soppela et al., 2007; Häikiö et al., 2007; Silfver et al., 2008; Appendix, Table 1). Only a few experiments have been conducted to study O_3 sensitivity of late-successional Finnish conifer species (Utriainen and Holopainen, 2001a,b). In these relatively short-term experiments with Scots pine (*Pinus sylvestris* L.) and Norway spruce, O_3 responses were mainly recorded as growth stimulations: average AOT40 exposure of 12 $\mu l\ l^{-1}$ h per growing season resulted in 0.2% reduction in stem height growth, whereas stem dry mass was increased by 21%, root dry mass by 12% and radial growth by 3% (averages for Scots pine and Norway spruce) as compared to control plants (Fig. 2; Utriainen and Holopainen, 2001a,b; Appendix, Table 1). Therefore, the results indicate that fast-growing pioneers such as *Betula* and *Populus* species are very sensitive to increasing O_3 concentration, while slow-growing conifer species such as *P. abies* and *Pinus sylvetris* are tolerant. However, longer experiments with conifers are still needed to confirm this conclusion. Nevertheless, there was also large genetic variation in O_3 sensitivity among the *B. pendula* and *Betula pubescens* clones in the Finnish investigations (Pääkkönen et al., 1997c). In that experiment 46 clones were screened for O_3 sensitivity in free-air conditions. A third of clones were ranked as sensitive, while another third were tolerant. High O_3 sensitivity of birches was related to thin leaves, high specific leaf area, high O_3 uptake, disturbed carbohydrate metabolism and failures in chloroplast membrane function (Kontunen-Soppela et al., 2007; Oksanen, 2003b; Oksanen et al., 2007). Large genetic variation in O_3 sensitivity has also been found among hybrid aspen (*P. tremula* × *P. tremuloides*) clones in Häikiö et al. (2009). In their study, accumulation of certain phenolic compounds (condensed tannins and catechins) was also linked to high O_3 tolerance.

Meteorology plays a key role in controlling O_3 entry into plants, and wind speed, temperature, vapour pressure deficit (VPD) are well known to be modified from ambient conditions even in large OTC in the field. In such systems, available plant space (branching, canopy size) is limited, nutrients and water are usually not limiting, and rooting space is constrained to varying degrees. In essence, essential processes inherent in forest ecosystems, such as natural water/energy flows and pest cycles are usually absent partly due to scale issues. The novel findings obtained from ecologically more relevant free-air field experimentation have shed critical light on the representativeness of previous chambered exposure studies, while offering new perspectives towards site-relevant risk analysis and extrapolation to the field situation.

3. O_3 sensitivity of climax trees

3.1. Knowledge on beech and spruce prior to free-air O_3 fumigation studies

Consensus prevailed towards the end of the 20th century within the scientific community that, based on chamber studies, chronic exposure to enhanced ground-level O_3 regimes has high injury potential for trees, including climax species like broad-leaf beech (*Fagus sylvatica* L.) and coniferous spruce (*P. abies*; Skärby et al., 1998; Matyssek and Innes, 1999). In particular, broad-leaf trees appeared to be sensitive, as reflected by declines in photosynthesis, premature leaf loss, increased dark respiration, and reduced biomass production (Matyssek et al., 1991, 1993; Pearson and Mansfield, 1993, 1994; Lippert et al., 1996a; Mikkelsen and Heide-Jörgensen, 1996; Langebartels et al., 1997; Matyssek and Sandermann, 2003). Although O_3 sensitivity was recognized to depend on O_3 uptake through stomata (Reich, 1987; Matyssek et al., 1995; Barnes and Wellburn, 1998; Lindroth et al., 1993), most O_3 risk assessments for forest trees were initiated on an O_3 exposure basis (Kärenlampi and Skärby, 1996; Fuhrer et al., 1997), focusing on chamber studies with – unlike field conditions – typically high coupling between O_3 exposure and O_3 flux (see section 2.1; Matyssek et al., 2007a,b). This is remarkable as both chronic O_3 impact and elevated CO_2 were known to reduce stomatal aperture (Ceulemans and Mousseau, 1994; Matyssek et al., 1995) although effects of O_3/CO_2 interactions have been little studied on climax trees. Unlike findings from herbaceous crop species (e.g. McKee et al., 1995, 1997; Mortensen, 1997; Reid and Fiscus, 1998), spruce has not displayed counteracting effects of elevated CO_2 on O_3-induced injury (Barnes et al., 1995; Lippert et al., 1996b, 1997; Sehmer et al., 1998), and only one experiment had addressed combined O_3/CO_2 effects on beech (Grams et al., 1999; Grams and Matyssek, 1999), the most important climax tree species in Central Europe (Ellenberg, 1996).

In a conventional phytotron study with potted saplings (Grams et al., 1999), pre-acclimation of juvenile beech for one year to elevated CO_2 resulted in lowered chlorophyll and nitrogen levels of leaves during the subsequent spring, while hardly affecting photosynthesis. Injury by elevated O_3 developed by mid-summer, as indicated by leaf necroses and decline in photosynthetic light and dark reactions. These occurred, in particular, under the ambient CO_2 regime. However, plants exposed to concurrently elevated CO_2 and O_3 levels maintained photosynthetic performance similar to individuals grown either under ambient CO_2 and O_3 combined, or elevated CO_2 and ambient O_3 conditions separately. High O_3 exerted stomatal closure at ambient but hardly at elevated CO_2, the latter overruling the O_3 impact while reducing stomatal width. Whole-plant biomass production reflected photosynthetic performance in each treatment (Kolb and Matyssek, 2001). In total, long-term exposure to elevated CO_2 counteracted adverse chronic O_3 effects in beech at the leaf and whole-plant level.

Evidence about negative O_3 impacts *per se* on current-year needles of 3 to 5-year-old *P. abies* saplings had been provided by several growth chamber studies (Havranek et al., 1990; Kronfuss et al., 1996, 1998; Wieser et al., 1998). Exposure to long-lasting mean O_3 concentrations above 100 nl l^{-1} induced significant decline in net photosynthetic capacity by 20–25% and reduction in stomatal conductance by 10–30% when compared to controls under O_3-free air (Havranek et al., 1990; Kronfuss et al., 1998). Decline was accompanied by reduction in carboxylation efficiency, suggesting carbon fixation as the primary site of O_3 injury, given the unaffected photochemical efficiency of photosystem II (Fv/Fm; Wieser et al., 1998). In parallel, increased dark respiration by 20% and decline in starch content (by up to 70%) indicated enhanced metabolic activity, suggesting allocation to detoxification and repair rather than storage. During the first five weeks of exposure, total and reduced ascorbate increased, but the antioxidative system was overwhelmed when cumulative O_3 uptake (COU) exceeded 6.1 mmol m^{-2} of total needle surface area.

Gradually increasing O_3 exposure from zero to 100 nl l^{-1} in weekly steps of 25 nl l^{-1} conversely yielded higher carbohydrate and antioxidant levels as compared to controls under O_3-free air (Wieser et al., 1998), seemingly promoting tolerance to O_3 stress. Such a kind of acclimation may explain the lack of O_3 effects in field-grown trees, where O_3 exposure typically changes less abruptly and is generally at lower levels than in many chamber experiments (Wieser et al., 1998). However, as underlined above for pioneer trees (sect. 2.1), knowledge about the O_3 responsiveness of climax trees has been restricted to (typically potted) saplings grown under chamber conditions, increasing the level of uncertainty for the transfer of findings to site conditions (including biotic pressure), and advanced life stages. Doubts were nourished by findings on the sensitivity to elevated CO_2 which was distinctly modified in the presence of competition between plants (Körner, 2006).

3.2. Novel phytotron studies: competition and pathogen effects on O_3 sensitivity

The 2-year phytotron study highlighted in Kozovits et al. (2005a,b) was unique in that for the first time effects of intra and inter-specific competition on tree sensitivity to combined O_3/CO_2 regimes were analyzed with climax species (saplings of *F. sylvatica* and *Picea abies*, 5 and 6 years old at the end of the study, respectively; cf. Ellenberg, 1996). In addition, uniqueness was conferred by reproducing above-canopy daily courses of PPFD with sunlight-similar PAR from Kranzberg Forest (see sect. 3.3; Payer et al., 1993, maximum phytotron PPFD of 900 μmol m^{-2} s^{-1}), along with the regimes of air and soil temperature, air humidity and ambient O_3 levels ($1 \times O_3$) as recorded throughout a previous growing season. Elevated O_3 and CO_2 treatments were $2 \times O_3$ (<150 nl l^{-1}) and ambient CO_2 + 300 μl l^{-1}, respectively, resulting in four O_3/CO_2 regimes.

When beech grew in monoculture, whole-plant biomass increment was about two to three-times higher as compared with growth in mixed culture with spruce, irrespective of gas regimes. The effect of mixture was exacerbated in beech by enhanced O_3. Spruce productivity contrasted by being higher in mixed than monoculture, profiting from the weakness of beech under high O_3. In summary, competition governed the productivity of both tree species, an outcome similar to that of competition studies under elevated CO_2 or O_3 (Körner, 2006; Poorter and Navas, 2003; Barbo et al., 1998, 2002; McDonald et al., 2002). The competitive weakness of beech relative to spruce was probably mediated through the acidic soil used in the phytotron study (cf. Körner, 2006; Spinnler et al., 2002). The weakness of beech in mixture was accompanied by a declining ability of efficiently occupying aboveground space through biomass investment, being a measure of plant competitiveness (Grams et al., 2002; Matyssek et al., 2005; Reiter et al., 2005; Grams and Andersen, 2007; Gayler et al., 2006), whereas in spruce, such an effect was absent (Kozovits et al., 2005a,b).

Also belowground, beech was less effective in mixture in competing for nitrogen (N), in particular, under elevated O_3 and CO_2, as reflected by decreases in N content and concentration at the whole-plant level (Kozovits et al., 2005a,b). Conversely, spruce in mixture displayed respective enhancements along with biomass increase. This belowground superiority of spruce was associated with limited water availability to beech, in particular under high O_3. Beech displayed isotopic carbon and oxygen signatures of enhanced WUE (mediated through stomatal closure) in the presence of spruce (Grams et al., 2007; Grams and Matyssek, 2010). Also an OTC study demonstrated competition between beech and a shrub pioneer species to modify extents of growth and foliar O_3 injury (Novak et al., 2008).

In the phytotron study by Luedemann et al. (2005, 2009), the focus was on mixed beech/spruce systems exposed to the two O_3 regimes in combination with controlled infection by the root rot pathogen *Phytophthora citricola*. The superiority of spruce in N acquisition was affirmed by an increased daily ^{15}N uptake per unit of fine-root biomass under 2 × O_3 and, remarkably, concurrent pathogen infection. The latter, when co-occurring with 2 × O_3, apparently enhanced the N acquisition capacity of spruce (Luedemann et al., 2005, 2009), which was substantiated by an increased dry-mass related N concentration at the whole-plant level. This effect perhaps indicated enhanced N demand of spruce in stress defence (Matyssek et al., 2005; Grams and Matyssek, 2010). Apparently, both tree species pursued different strategies in coping with oxidative stress, whether exerted by O_3 or the pathogen (cf. Matyssek and Sandermann, 2003): Chronic O_3 impact preceding the infection had probably hardened beech against the pathogen, so that further decline in biomass production was prevented when both oxidative stressors acted in concert (cf. Luedemann et al., 2005, 2009). In spruce, hardening did not occur, and there was a distinct decline in productivity along with an increase in N acquisition under concurrent 2 × O_3 and pathogen impact.

In summary, beech and spruce did not perform in terms of their respective O_3 and CO_2 sensitivities as one would have concluded from previous chamber studies, when trees were typically grown in isolation (i.e. as potted individuals, Matyssek and Sandermann, 2003). Intra and inter-specific competition determined, depending on the species, O_3 and CO_2 sensitivity. Pathogen impact underlines the importance of biotic stress, increasing the degree of uncertainty about tree responsiveness.

3.3. Performance of adult beech/spruce under enhanced O_3 stress

Knowledge on the O_3 sensitivity of adult forest trees has been limited by the scarcity of experimental analysis under actual stand conditions (Matyssek and Innes, 1999), deficits in whole-tree approaches (Matyssek and Sandermann, 2003), and lack of controls in the field (e.g. Baumgarten et al., 2000). Beyond the use of branch cuvettes or bags mounted into tree crowns (Havranek and Wieser, 1994; Houpis et al., 1991), or of large chambers for whole-tree O_3 fumigation (Hanson et al., 1994), which altogether led to concerns about micro-climatic and, as a result, biochemical artefacts in relation to non-enclosed plant parts (Matyssek et al., 1997; Sandermann et al., 1997; Henriksson, 2001), free-air fumigation systems were recognized as the ultimate choice for approaching an ecologically relevant O_3 stress analysis (Musselman and Hale, 1997; Karnosky et al., 2001). Such an approach had been employed to forest trees of the climax species *A. saccharum* Marsh restricted, to clusters of sun and shade foliage (Tjoelker et al., 1995). The only whole-tree free-air O_3 fumigation experiment conducted to date on climax species was performed in a mixed beech/spruce stand (*Fagus sylvatica/P. abies*, Kranzberg Forest, Freising/Germany; trees of 27 m height, about 60 years old) through the CASIROZ case study (*"Carbon Sink Strength of Fagus sylvatica L. in a Changing Environment – Experimental Risk Assessment of Mitigation by Chronic Ozone Impact"; Matyssek et al., 2007a)*. Novel exposure methodology was employed, based on rows of vertically suspended teflon™ tubes, expanding across the foliated stand canopy for O_3 release (details on design in Werner and Fabian, 2002; Nunn et al., 2002; Karnosky et al., 2007b). In relation to the unchanged ambient O_3 regime of the forest site (i.e. 1 × O_3 defined as control), the experimental enhancement was 2 × O_3 (O_3 levels restricted to < 150 nl l^{-1}: Nunn et al., 2002; Matyssek et al., 2007a). 1 × O_3 and 2 × O_3 provided the target regimes of the beech/spruce phytotron study outlined in section 3.2 (Kozovits et al., 2005a,b; Luedemann et al., 2005, 2009). In the following section, the whole-tree free-air O_3 fumigation approach will be highlighted. The free-air fumigation was operated during the growing seasons of an 8-year period (i.e., 2000–2007). Emphasis was on beech, although O_3 responses of spruce will be addressed to the extent investigated.

3.3.1. Experimental free-air O_3 canopy exposure at Kranzberg Forest

A spectrum of leaf level responses demonstrated O_3 impact in beech on metabolism after exposure to 2 × O_3 (Matyssek et al., 2007a). Gas exchange displayed decreased stomatal conductance at unchanged carboxylation capacity and mesophyll conductance for CO_2 (Warren et al., 2007), so that photosynthesis was mainly limited through reduced Ci upon O_3-induced stomatal closure (Kitao et al., 2009). Only shade leaves showed accelerated senescence under 2 × O_3 (cf. Gielen et al., 2007). In spruce, photosynthesis was limited by 2 × O_3, decreasing during the first year of exposure (Nunn et al., 2006).

Findings in beech were consistent with enhanced $\delta^{13}C$ of leaf organic matter across sun and shade foliage under 2 × O_3 (Kitao et al., 2009) indicating increased photosynthetic WUE (Farquhar et al., 1989). Such a conclusion is supported by lowered rather than increased PEPc activity under 2 × O_3 (Blumenröther, pers. comm.; see sect. 2). This contrasts with findings from juvenile trees in previous chamber studies (Saurer et al., 1995), although enhanced respiratory activity suggested increased energy demand for stress defence in the adult trees (Kitao et al., 2009). Drought dominated the O_3 effect on stomata by inducing closure as observed in 2003 (Löw et al., 2006), the year of the extraordinarily dry summer conditions in Central Europe (Ciais et al., 2005). Stomatal closure decoupled O_3 exposure from uptake, as the enhanced cumulative exposure relative to that of humid years decreased by about 55% and 40% at 2 × O_3 and 1 × O_3, respectively. It was associated with only a slight increase in O_3 uptake under 2 × O_3, and an even lower uptake at 1 × O_3 (Löw et al., 2006). Drought had the capacity of altering the metabolic sensitivity per unit of O_3 uptake (i.e. the effective O_3 dose, Matyssek et al., 2008), as indicated by relationships between O_3 uptake and photosynthesis that differed between 2003 and humid summer conditions (Löw et al., 2007). Summer conditions and tree performance in 2003 underline the paramount importance of considering drought when calculating actual O_3 uptake and sensitivity (Matyssek et al., 2006, 2008).

2 × O_3 reduced levels of sucrose and starch in beech leaves regardless of summer conditions (Blumenröther et al., 2007), perhaps reflecting, in the case of starch, raised repair and detoxification demand similar to findings about O_3-stressed juvenile pioneer trees of controlled chamber studies (cf. Landolt et al., 1997; Einig et al., 1997; see sect. 2). In parallel, levels of the reduced forms of the antioxidants glutathione and ascorbate (two central components of detoxification) were decreased (Haberer et al., 2007). Metabolic O_3 responses were associated with signaling of oxidative stress and gene expression (Jehnes et al., 2007), which through induction of the NCED1 gene, responsible for stimulated ABA synthesis under enhanced O_3 uptake, appeared to relate to the decline of stomatal conductance under 2 × O_3 (Löw et al., 2006, 2007; Kitao et al., 2009). Also, hardening by 2 × O_3 against

pathogenic infection was seen in adult beech (Bahnweg et al., 2005; cf. sect. 3.2), here mediated through the endophyte *Apiognomonia errabunda*, which can cause beech blight disease. During the summer, the degree of leaf infection remained lower under 2 × O_3 than under 1 × O_3 (Bahnweg et al., 2005).

Remarkable were the phytohormonal relationships under 2 × O_3. Contents of active cytokinin (CK) were lower in beech leaves and phloem sap under 2 × O_3, whereas levels were markedly enhanced in fine roots and xylem sap relative to 1 × O_3 (Winwood et al., 2007). This means, an O_3-associated increase in CK destruction in leaves resulted in a decreased export of mobile forms of isopentenyladenine-type CK to the roots, with the expected consequence: diminished CK-mediated suppression of fine-root growth (Winwood and Pate, 2007; cf. Riefler et al., 2006). The resulting enhanced fine-root growth in turn fostered mycorrhization (Grebenc and Kraigher, 2007) as a consequence of the altered phytohormonal shoot-root communication due to above ground O_3 stress (cf. Andersen, 2003). In turn, the increase in developmental activity in roots promoted the on-site synthesis of CKs, chiefly zeatin ribotide - the inactive, non-mobile precursor of the transport form, zeatin riboside, exported from roots in the xylem. Finally, the enhanced levels of xylem CK arriving in the leaves of 2 × O_3-treated trees are likely to mitigate the leaf senescence-accelerating effect of O_3-associated CK destruction, an explanation for why beech leaves do not readily show symptoms of chronic exposure to elevated O_3.

In particular under humid summer conditions, soil respiration rate was enhanced under 2 × O_3 both underneath beech and spruce trees (Nikolova et al., 2009, 2010), corroborating similar findings of other studies (Andersen and Scagel, 1997; Pregitzer et al., 2006). However, only in beech fine-root production was stimulated under 2 × O_3 and soil respiration stayed enhanced during the drought of 2003. Conversely, fine-root production in shallow-rooted spruce was reduced by summer drought (Nikolova et al., 2009, 2010). In both species, drought had the capacity to overrule stimulating O_3 effects on fine-root turnover and soil respiration. In general, spruce roots showed low morphological and physiological plasticity under 2 × O_3 compared to beech (Nikolova pers. comm.). Spruce was not less O_3-sensitive belowground than beech. In total, extents of above and belowground O_3 responses varied spatio-temporally, i.e. between years, within growing seasons and – above ground – between sun-exposed and shaded crown positions, in beech and in spruce (Nunn et al., 2005; Matyssek et al., 2007a). Macroscopic O_3-induced leaf injury, however, only occurred in beech, being negligible under drought due to its mitigating effect on stomatal opening and O_3 uptake (Löw et al., 2006).

Given the variability in O_3 sensitivity, what were the consequences for stem growth in both tree species? Wipfler et al. (2005) had reported some O_3-caused decline in the radial stem growth of spruce, but no such decline in beech, when evaluating a three-year observation period. Their analysis was based on annual ring growth at breast height, representing the conventional assessment approach in yield science. When accounting for the diameter-height growth allocation, i.e. assessing the annual increment of the entire stem volume, and hence, total stem mass, however, a different outcome emerged (Pretzsch et al., 2010): While over the total free-air O_3 fumigation experiment of eight consecutive years, growth of both spruce and beech in stem diameter at breast height was reduced by 12% each rather than in stem height (a similarity to findings in pioneers of chamber studies, see section 2), the resulting volume growth increased by 2% in spruce, but decreased by 40% in beech in relation to the respective 1 × O_3 trees (Pretzsch et al., 2010). Upon scaling this outcome to the stand level, an increase in annual volume growth of 0.5 $m^3\ ha^{-1}$ in spruce under 2 × O_3, but a loss of 10 $m^3\ ha^{-1}$ in beech became apparent. In view of this whole-stem approach of annual stem production, in particular beech supports conclusions by Sitch et al. (2007) based on modelling that chronic O_3 stress, acting as a factor in climate change, has the capacity of substantially lowering the carbon sink strength of trees.

4. Comparison pioneer *versus* climax species under harsh site conditions

The physiological sensitivity of adult field-grown *P. abies*, *P. cembra* (both being climax species) and *L. decidua* trees (pioneer species) to O_3 has been examined in a limited number of controlled field studies at the timberline ecotone on Mt. Patscherkofel (1950 m a.s.l.). These studies made use of climatized cuvettes for exposing twigs attached to tree crowns to defined O_3 regimes (Havranek et al., 1989; Volgger, 1995; Wieser et al., 2001). Following 12 weeks of exposure to mean O_3 levels between 0 and 100 nl l^{-1}, no distinct treatment effects were detected in net photosynthetic capacity (A_{max}) of *P. abies* (Havranek et al., 1989) and *P. cembra* (Wieser et al., 2001). The eight-week studies of Havranek and Wieser (1993) at a mountainous forest site (1000 m a.s.l.) did not report significant differences in A_{max} of *L. decidua* twigs under ambient or twice-ambient O_3 concentrations in relation to twigs under O_3-free air. These outcomes resembled those reported by Thornton et al. (1994), in that the absence of O_3 did not positively affect photosynthesis relative to twigs under O_3 exposure. Long-term exposure to mean O_3 concentrations permanently persisting above 100 nl l^{-1} however, had the capacity of affecting conifers at the timberline ecotone (Wieser and Havrenek, 2001). By adding 120 nl O_3 l^{-1} continuously to O_3-free air for 12 weeks, O_3 significantly reduced A_{max} by 30% as compared to controls under O_3-free and ambient air (Havranek et al., 1989). Similar reductions in A_{max} were observed in *L. decidua* after three to six weeks of exposure to 150 or 200 nl O_3 l^{-1}, respectively (Volgger, 1995; Wieser and Havranek, 1996). However, these concentrations were distinctly elevated relative to those of ambient conditions.

A threshold level relative to COU eliciting statistically significant reductions in A_{max} can be determined from boundary analysis (Wieser and Havranek, 2001). The boundary line in Fig. 4 (top) represents the threshold below which the combination of exposure duration and COU did not cause statistically significant reductions in A_{max} ($p < 0.01$) when compared to controls in O_3-free air. Incipient effects only occurred when COU exceeded 8.6 and 30.0 mmol m^{-2} of total needle surface area in *L. decidua* and *P. abies*, respectively (Fig. 4, top).

As COU is a quantitative measure of O_3 impact, focus was directed also to the rate-based O_3 flux (FO_3, Fig. 4, bottom). FO_3 needed to approach 0.41 and 0.47 nmol $m^{-2}\ s^{-1}$ of total needle surface area after 12-week exposure in the evergreen climax species *P. abies* and 3-week exposure in the deciduous pioneer species *L. decidua*, respectively, to elicit statistically significant effects on A_{max}. Such FO_3 "thresholds" are two to four times above the mean seasonal FO_3 at the timberline assessed for adult trees of *P. abies* (0.16 nmol $m^{-2}\ s^{-1}$), *P. cembra* (0.11 nmol $m^{-2}\ s^{-1}$) and *L. decidua* (0.22 nmol $m^{-2}\ s^{-1}$) under ambient O_3 regimes (Wieser et al., 2000, 2002; Wieser and Havranek, 1995). Although the deciduous pioneer larch seems to be more sensitive to elevated O_3 than the evergreen climax species spruce and cembran pine, chronic O_3 exposure *per se* presently does not represent a dominating stress on conifers in the timberline ecotone of the Central European Alps (Wieser et al., 2008, 2009).

Fig. 4 reflects the consistency between deciduous pioneer and evergreen climax tree species exposed to elevated O_3 concentrations >120 nl l^{-1}. These data also indicate species-related differences in O_3 susceptibility not solely attributable to differences in

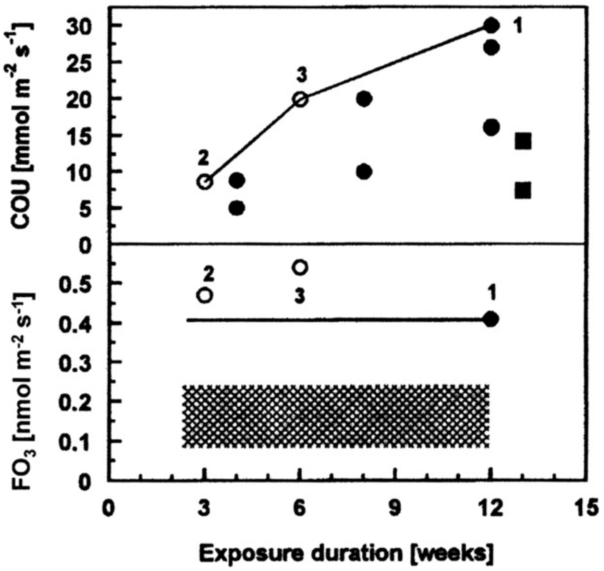

Fig. 4. Boundary line analysis representing the relationship between the combination of exposure duration and cumulative O_3 uptake (COU: top) as well as relationship between the combination of exposure duration and O_3 flux (FO_3; bottom) and significant reductions in net photosynthetic capacity at ambient CO_2 concentration (A_{max}) of adult *Picea abies* (closed circles), *Pinus cembra* (closed squares), and *Larix decidua* trees (open circles) at the alpine timberline. The boundary line defines the threshold below which the combination of exposure time and COU or FO_3 did not significantly affect A_{max} compared to O_3-free and ambient air controls. Mean O_3 concentrations eliciting statistically significant reductions ($p < 0.01$) were (1) 120 nl l^{-1}, (2) 150 nl l^{-1}, and (3) 200 nl l^{-1}. The dotted band represents the mean average FO_3 estimated for adult conifers under ambient O_3 concentrations at timberline. Compiled after data from Havranek et al. (1989), Volgger (1995), Wieser and Havranek (1996, 2001), and Wieser et al. (2001).

FO_3. Factors like maintenance and defence appear to be important as well (Matyssek et al., 2008). Although detoxification is suggested to be less energy demanding than repair (Musselman and Massman, 1999) there is a lack of data on the extent of repair and on the control of carbon allocation between both metabolic pathways counteracting O_3 stress.

5. Conclusions

A broad spectrum of investigations on the O_3 sensitivity of trees was compiled during the second half of the 20th century. However, this literature has revealed a number of issues (cf. Kolb and Matyssek, 2001) related to limitations in experimental methodology: (1) plant growth mostly occurred under controlled but chamber conditions with the potential of biasing micro-climate and, as a consequence, plant response to O_3; (2) often non-fluctuating (square wave) and excessively high O_3 exposures were used along with intense air-mixing that enhanced O_3 uptake and injury; (3) most experiments were restricted to juvenile tree stages; (4) restriction of root growth in pots likely biased whole-plant resource allocation and performance; (5) often, non-limiting water and nutrient supply were featured promoting O_3 uptake, thus, enhancing sensitivity; (6) due to their size, chambers excluded multi-species assemblages and other biotic agents (pathogens, herbivory, mycorrhization); and (7) O_3 exposure times were limited, mostly shorter than three growing seasons.

These issues have been identified by the scientific community (cf. Manning, 2005) and have led to a recognition that, although the science was informative around mechanism of response and genetic screening for sensitivity, the knowledge gained was more limited in predicting tree performance under chronic O_3 stress at actual forest sites with their multi-factorial stress scenarios (Matyssek and Innes, 1999). It took until the end of the 20th century that advanced phytotron technology (Payer et al., 1993) permitted distinguishing, through a controlled approach, tree responsiveness to combined O_3/CO_2 regimes from competitive and pathogenic impact in climax trees. One significant step at the beginning of the 21st century towards gathering experimental evidence under ecosystem conditions was, however, the introduction of the free-air O_3 fumigation approach to studying whole trees growing in stands over many years (Karnosky et al., 2001). Nevertheless, this approach also has its restrictions, such as the inability to control O_3/CO_2 concentrations below ambient levels, which are above those of pre-industrial times. Hence, risk potential of enhanced O_3 and CO_2 regimes need to be concluded from differential tree responsiveness to enhanced and ambient regimes, such as future comparison estimating tree susceptibility under present conditions. Other experimental limitations are in fact being overcome, such as the avoidance of micro-climatic bias, while permitting whole-plant assessment of tall trees in maturing stands, as well as the presence of the prevailing, abiotic and biotic multi-factorial site scenario. Recent experiments of this latter kind, covering long-term tree growth (up to one decade) in plantations and forests in Europe and the US, focusing on pioneer and climax species, have been highlighted here under one common perspective. In our assessment, we conclude that the key science messages include:

1) In agreement with the highlighted novel phytotron and earlier chamber studies, enhanced O_3 regimes have the capacity of counteracting effects by elevated CO_2 and of substantially reducing the carbon sink strength of woody-plant systems.
2) However, degree and direction of effect is strongly governed by the genotype, competing species life histories and ontogeny rather than by the tree species *per se*.
3) Complexity (and unpredictability) in response is increased when pathogens or herbivores are involved, as their presence is not only conducive to injury development under enhanced O_3, but the latter can favour hardening against consumers.
4) Pioneer tree species tend to be more susceptible to O_3 impact than climax species, as indicated even under the harsh growth conditions of the timberline ecotone. However, the complexity of interactions between genotype, competition, host-pest association, ontogeny, site conditions and pre-history can hinder generalization about O_3 responsiveness in view of the successional status of a tree species.
5) Site conditions that may be conducive to hardening against O_3 stress or increasing susceptibility, i.e. to altering the “effective O_3 dose” (Matyssek et al., 2008), demand for particular attention.
6) Adult climax trees growing at steady-state forest conditions under experimental free-air O_3 exposure were no exceptions in terms of physiological/biochemical sensitivity, decline in productivity and endophytic/pathogenic interaction under chronic O_3 stress, although response patterns amongst tree parameters and years were highly variable.

In summary, this new evidence does not conflict with knowledge on basic tree responses to chronic O_3 stress represented in findings from previous controlled chamber studies. Rather, we have gained important new knowledge, such as about the strong modifying impact of biotic agents (competition, genotype, ontogeny in the presence of natural mycorrhization). Previous evidence provided principles in O_3 response, however, with limited ecological significance (cf. Schaub et al., 2005). Fig. 5 underlines the complementary quality of chamber and free-air approaches, with the latter representing a methodological advancement by enhancing the ecological relevance of experimentation outcome in

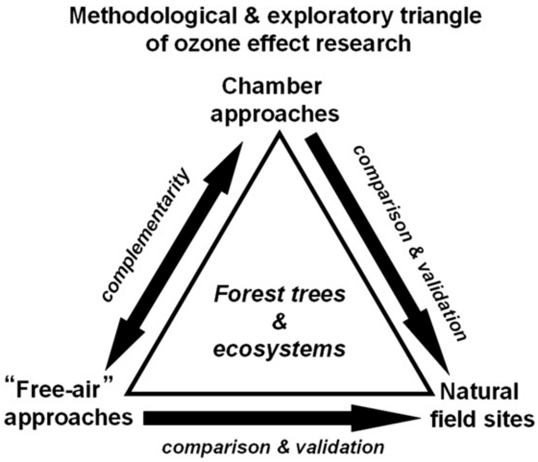

Fig. 5. Methodological and exploratory triangle of O_3 effect research in forest trees and ecosystems; free-air O_3 fumigation approaches, possessing complementary quality in relation to chamber approaches, enhance the ecological relevance of experimentation outcome in view of natural site conditions. Both approaches require synthesis towards up-scaling to and validation at natural field sites.

terms of more realistic biotic and abiotic quality. Still, both approaches require validation at natural field sites. Having gained new evidence from free-air O_3 fumigation experiments, insights now exist into tree growth beyond the juvenile stage under chronic O_3 stress in plantations and in established forests. Nevertheless, the predictability of tree performance under any scenarios, comprising enhanced O_3 impact amongst other stressors, remains a challenge, because of the variability in spatio-temporal associations of tree responses that represent an integration of the modulating effects of biotic and abiotic interactions. The ultimate step in completing the methodological and exploratory triangle of O_3 effect research in forest trees and ecosystems as illustrated in Fig. 5 – i.e. validation – will continue to remain a central task in future research. For achieving this task, the factorial complexity demands for unravelling underlying mechanisms, including the molecular basis, which need to be explored in tree species within a site-relevant context rather than – as still largely being the case – in model plant systems (Matyssek et al., 2005, 2008; Sandermann and Matyssek, 2004). This is another key message to be learnt from the recent phytotron and free-air O_3 exposure studies summarized in this review.

In the view of this assessment, we conclude that our mechanistic understanding of cause/effect-related complexity within actual ecophysiological, i.e. site-relevant, contexts is still rather poor. We are only barely able to establish relationships between tree performance and O_3 uptake rather than exposure, and we have little knowledge on the mechanisms determining the "effective O_3 dose", i.e. sensitivity per unit of O_3 uptake, that governs tree performance under actual field scenarios (Matyssek et al., 2008). Overcoming this deficit must be taken seriously, as the novel evidence underlines that enhanced O_3 regimes do have the capacity of mitigating the carbon sink strength of trees and woody-plant systems (Fowler et al., 2008; Sitch et al., 2007; Dentener et al., 2006). And last but not least, one key message obtained from this novel work is that the free-air O_3 fumigation methodology offers an advanced tool for attaining the ecosystem level in O_3 cause/effect analysis (European Science Foundation, 2009). This remaining research need is crucial for realistically assessing and counteracting risks associated with enhanced O_3 regimes and climate change scenarios.

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Appendix

Table 1

Ozone caused changes in main growth responses (foliage area, stem height increment, dry masses when available, radial growth) of Finnish tree species calculated as mean values for all genotypes (both sensitive, intermediate and tolerant pooled together) and the whole exposure time. All data are derived from experiments where optimum fertilization and watering treatments were used (i.e. low N, high N, drought and wet treatments were excluded) *Stem base diameter; **Shoot = stem + leaves.

Species	Total AOT40 exposure, ppm h (average per growing season)	Exposure facility	Duration, potted/ soil-growing	Average change (+/−) in growth, %	Reference
Betula pendula	34.2	Growth chamber	25 days, potted	Foliage area −17% Stem height −5%	Pääkkönen *et al.* 1995 (Table 1)
Betula pendula	21.8	Growth chamber	36 days, potted	Foliage area −10% Stem DWT −1.3% Root DWT −10%	Pääkkönen*et al.* 1998 (Table 2; well watered plants)
Betula pendula	14.0	Growth chamber	20 days, potted	Stem heigh −7% Stem DWT −8%	Oksanen & Holopainen 2001 (Table 1)
Betula pendula	75.9 (25.3)	Open-top chamber	Three growing seasons, soil-growing	Foliage area −17% Stem height −11% Stem DWT −9% Root DWT −23% Radial growth −12%	Riikonen *et al.* 2004 (Fig 1, 3; Table 3)

Table 1 (*continued*)

Species	Total AOT40 exposure, ppm h (average per growing season)	Exposure facility	Duration, potted/ soil-growing	Average change (+/−) in growth, %	Reference
Betula pendula	9.2	Free-air fumigation	One growing season. potted	Foliage area −10% Stem height +13%	Pääkkönen & Holopainen 1995 (Table 2; medium N)
Betula pendula	9.2	Free-air fumigation	One growing season, potted	Foliage area −6% Stem height +18% Stem DWT −9% Root DWT −14%	Pððkk—nen & Holopainen 1995 (Table 3; medium N)
Betula pendula	1.7	Free-air fumigation	One growing season. potted	Foliage area −7% Stem height +15% Stem DWT −2%	Pääkkönen*et al.* 1996 (Table 2)
Betula pendula	17.3	Free-air fumigation	One growing season,	Foliage area −23%	Oksanen & Saleem 1999
Betula pendula	101.3 (14.5)	Free-air fumigation	Seven growing seasons	Stem DWT −48% Root DWT −41%	Kontunen-Soppela *et al.* 2007 (Fig 1)
Populus tremuloides × *P. tremula*	23.0	Growth chamber	32 days, potted	Stem height −6% Stem DWT −13% Root DWT −31%	Oksanen *et al.* 2001 (Table 2)
Populus tremuloides × *P. tremula*	42.1	Growth chamber	32 days, potted	Stem height −39% Stem DWT −53% Root DWT −58%	Oksanen *et al.* 2001 (Table 2)
Populus tremuloides × *P. tremula*	1.6	Free-air fumigation	Two months, potted	Stem height +13% Stem DWT +0.3% Root DWT −10%	Oksanen *et al.* 2001 (Table 2)
Populus tremuloides × *P. tremula*	34.3 (17.2)	Free-air fumigation	Two growing seasons, potted	Stem height −20% Stem DWT −5% Root DWT −20% Radial growth −17%	Häikiö *et al.* 2007 (Fig 4, 5; Table 4; Averaged for low-N and high-N treatments)
Populus tremuloides × *P. tremula*	44.7 (14.9)	Free-air fumigation	Three growing seasons	Foliage area −19% Stem DWT −19% Root DWT −6% Radial growth −7%	Häikiö *et al.* 2009
Populus tremula	34.3 (17.2)	Free-air fumigation	Two growing seasons, potted	Stem height −16% Stem DWT −11% Root DWT −18% Radial growth −18%	Häikiö *et al.* 2007 (Fig 4, 5; Table 4; Averaged for low-N and high-N treatments)
Picea abies	21.9 (11.0)	Free-air fumigation	Two growing seasons, potted	Stem height−0.4% Stem DWT +37% Root DWT +18% Radial growth +0.7%	Utriainen and Holopainen 2001a (Table 4, 5; control fertilization level)
Pinus sylvestris	37.0 (12.3)	Free-air fumigation	Three growing seasons, potted	Stem height +0.0% Stem DWT +5% Root DWT +7% Radial growth +6%	Utriainen and Holopainen 2001b (Fig 1; Table 3; control fertilization level)
			potted	Stem DWT −17% Root DWT −19% Radial growth* − 9%	(Table 3)
Betula pendula	8.3 (5.5)	Free-air fumigation	One and half growing seasons potted	Stem height +2% Radial growth −33% Stem DWT +17% Root DWT +11%	Silfver *et al.* 2008 (Table 3)
Betula pendula	27.2 (13.1)	Free-air fumigation	Two growing seasons, potted	Foliage area −9% Stem height −6% Stem DWT +2%	Pääkkönen & Holopainen 1995 (Table 2; medium N)
Betula pendula	36.9 (18.5)	Free-air fumigation	Two growing seasons, potted	Foliage area −9% Stem height +1.6% Stem DWT −7%	Pääkkönen *et al.* 1997a (Table 2)
Betula pendula	36.9 (18.5)	Free-air fumigation	Two growing seasons, potted	Foliage area −14% Stem height +0.1% Stem DWT −9%	Pääkkönen *et al.* 1997a (Table 2)
Betula pendula	34.7 (17.4)	Free-air fumigation	Two growing seasons, potted	Foliage area +4% Stem height −4% Stem DWT −11% Root DWT +13%	Pääkkönen *et al.* 1997b (Table 3)
Betula pendula	31.2 (15.6)	Free-air fumigation	Two growing seasons, potted	Shoot** DWT −13% Root DWT −54%	Saleem *et al.* 2001 (Table 3)

(*continued on next page*)

Table 1 (*continued*)

Species	Total AOT40 exposure, ppm h (average per growing season)	Exposure facility	Duration, potted/ soil-growing	Average change (+/−) in growth, %	Reference
Betula pendula	41.4 (12.9)	Free-air fumigation	Three growing seasons	Stem height −3% Radial growth +0.02%	Saleem *et al.* 2001 (Table 3)
Betula pendula	75.0 (15.0)	Free-air fumigation	Five growing seasons, potted	Stem height +3% Shoot DWT −21% Root DWT −34%	Oksanen 2001 (Table II)
Betula pendula	87.1 (14.5)	Free-air fumigation	Six growing seasons; soil-growing	Foliage area −30% Stem height −5% Radial growth −15%	Oksanen 2003a (Fig 3; Table 2)
Betula pendula	87.1 (14.5)	Free-air fumigation	Six growing seasons, soil-growing	Foliage area −36% Stem height −15% Radial growth −20%	Oksanen 2003b (Fig 2, 4)

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