New flux based dose–response relationships for ozone for European forest tree species

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ABSTRACT

To derive O₃ dose–response relationships (DRR) for five European forest trees species and broadleaf deciduous and needleleaf tree plant functional types (PFTs), phytotoxic O₃ doses (POD_y) were related to biomass reductions. POD_y was calculated using a stomatal flux model with a range of cut-off thresholds (y) indicative of varying detoxification capacities. Linear regression analysis showed that DRR for PFT and individual tree species differed in their robustness. A simplified parameterisation of the flux model was tested and showed that for most non-Mediterranean tree species, this simplified model led to similarly robust DRR as compared to a species- and climate region-specific parameterisation. Experimentally induced soil water stress was not found to substantially reduce POD_y, mainly due to the short duration of soil water stress periods. This study validates the stomatal O₃ flux concept and represents a step forward in predicting O₃ damage to forests in a spatially and temporally varying climate.

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1. Introduction

A large body of evidence has shown that ozone (O₃) causes damage to trees (Wittig et al., 2009; Matyssek et al., 2010). These O₃

effects range from impacts such as visible injury on foliage (Schaub, 2005), decreasing leaf chlorophyll content and photosynthesis (Wittig et al., 2009), changes in carbon allocation (Paoletti et al., 2009) and biomass production (Wittig et al., 2009), premature leaf senescence (Pell et al., 1999), and altered tree water use (Sun et al., 2012). By synthesising information expressed as O₃ flux based dose–response relationships (DRR) derived from field-experiments, critical levels (CLs) have been identified above which O₃ damage would be expected to occur (LRTAP Convention, 2010; Mills et al., 2011). The United Nations Convention on Long-Range Transboundary Air Pollution (LRTAP Convention) has used such CLs as a policy tool to identify areas of CL exceedance across Europe and subsequently to formulate European emission reduction strategies to improve air quality.

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This study presents the next stage in the derivation of DRR for forest trees, which use the accumulated stomatal O₃ flux above a threshold 'y' as the dose metric (Embersson et al., 2007), often referred to as PODy (Phytotoxic Ozone Dose above a threshold 'y'), and the relative change in annual whole tree biomass production as the response metric (Karlsson et al., 2007). Current CLs for forest trees are based on an analysis performed by Karlsson et al. (2007) on Norway spruce (27 data points from 3 countries and 8 experiments) and beech/birch (38 data points from 3 countries and 14 different experiments) and were set to values for which there was a >95% confidence of finding a significant effect at the percentage loss chosen (LRTAP Convention, 2010; Mills et al., 2011). Since the publication of Karlsson et al. (2007), additional experimental data have become available that extend the species under investigation and increase the range of environmental conditions under which the fumigation or filtration experiments were performed. In addition, new methods to assess the influence of soil moisture on stomatal O₃ flux have been developed (Büker et al., 2012).

In performing this re-analysis including all currently available forest tree data, we addressed the following sources of uncertainty in flux-effect modelling for forest tree species: i. the parameterisation of the stomatal flux model; ii. the choice of the 'y' threshold (which is considered to statistically represent the plants' ability to detoxify a certain level of O₃ dose (Pleijel et al., 2007)); iii. the influence of reduced water-availability on stomatal O₃ flux and; iv. whether particular groupings of species, e.g. according to plant functional types (PFTs), with similar O₃ sensitivities can be identified. We also compared regression functions based on the same updated dataset using the updated flux methodology with those using the formerly accepted concentration-based approach (AOT40¹).

To investigate the parameterisation of the flux model, this study applied two different methods to estimate stomatal O₃ fluxes. The first method was that used previously by Karlsson et al. (2007), but with updates to include new parameterisations defined in the 2010 revision of the UNECE Mapping manual (LRTAP Convention, 2010). These 'real species' parameterisations incorporate new data to define parameter values and also identify climate specific parameterisations to account for different species ecotypes. A second method tested the suitability of a simplified parameterisation, i.e. standard functions that describe the effect of light, vapour pressure deficit and temperature on stomatal conductance (g_s) irrespective of species, called simplified parameterisation or simple model from hereon. Application of this method was used to test the hypothesis that a simplified parameterisation of the multiplicative g_s model leads to a similarly robust DRR as compared to the 'real species' parameterisation. As such it examined whether there is a need for a rigorous species-specific parameterisation of the model, which often complicates large-scale application of the flux based method both for flux-effect as well as total O₃ deposition estimation.

This study also presented the opportunity to compile new dose–response functions and datasets for two species (i.e. Holm oak and poplar) not considered in Karlsson et al. (2007). This allowed a more thorough investigation of the uncertainty in the current CLs related to the selection of the 'y' threshold. Karlsson et al. (2007) used a single 'y' value of 1.6 nmol O₃ m⁻² s⁻¹ based on a previous analysis (Karlsson et al., 2004), which found that this value gave higher R² values for flux–effect relationships when compared with thresholds of 0.0, 3.2 and 4.8 nmol O₃ m⁻² s⁻¹. Since it is more statistically robust to test a number of 'y' thresholds (Feng et al., 2012), the analysis presented here set out to trial a far wider and incrementally refined number of 'y' threshold values than has

been performed previously for forest tree species.

Another key area of uncertainty investigated here is the influence of reduced water availability on PODy and hence O₃ sensitivity. Some of the new datasets are derived from experimental O₃ fumigations or filtrations conducted under varying levels of water supply. This allows the effect of reduced water availability on PODy to be investigated using a new soil water balance method described in Büker et al. (2012). This is particularly important as the exclusion of data representing non-optimal water supply, which is known to reduce g_s (Büker et al., 2012) and therefore stomatal O₃ flux, has been cited as reason why the stomatal flux approach sometimes does not provide a substantial improvement of DRR developed using the concentration based AOT40 index (e.g. Karlsson et al., 2007).

Finally, this study also investigated whether species groupings of O₃ sensitivity can be defined. Past studies have been rather inconclusive in defining DRR that are able to represent different tree PFTs. For example, Karlsson et al. (2004, 2007) found Norway spruce, Scots pine and birch/beech to be more sensitive than Aleppo pine and oak such that no obvious distinction could be made between broadleaf and needleleaf trees. In contrast, a meta-analysis performed for forest trees by Wittig et al. (2009) found evidence for gymnosperms being less sensitive than angiosperms when related to O₃ concentrations. Also, broadleaf evergreen species have been reported to be more resistant to O₃ than broadleaf deciduous species (Calatayud et al., 2010, 2011; Zhang et al., 2012). However, a study by Reich (1987) suggested that when taking into account O₃ uptake (or flux) per leaf life span, conifers and hardwoods have similar sensitivity in terms of declines in photosynthesis and growth. This analysis provided an opportunity to investigate these issues in further detail using more advanced methods for estimating stomatal O₃ flux.

The overall aim of this study was to evaluate the performance of PODy and AOT40 metrics in predicting biomass reductions for forest trees. This was achieved through analysis of the statistical performance of linear regressions of DRR constructed using data collected from fumigation and filtration studies. This work was conducted with a view to developing new DRR that could be used in the derivation of critical levels within the LRTAP Convention.

2. Materials and methods

2.1. Estimating stomatal conductance g_s

The stomatal conductance (g_s) algorithm of the DO₃SE (Deposition of O₃ for stomatal exchange) model was used as the basis for estimates of g_s for all model runs. The model employs a multiplicative algorithm, based on that first developed by Jarvis (Jarvis, 1976), modified for O₃ flux estimates (Embersson et al., 2000a, 2000b, 2001, 2007; Büker et al., 2012) to estimate leaf/needle g_s (the inverse of r_s (stomatal resistance)) as:

$$g_s = g_{max} * f_{phen} * f_{PPFD} * \max \{f_{min}, f_T * f_D * f_{SW}\} \quad (1)$$

where the species-specific maximum g_s (g_{max}) is modified by relative response functions (scaled from 0 to 1) to account for g_s variation with leaf/needle age over the course of the growing season (f_{phen}) and the functions f_{PPFD} , f_T , f_D and f_{SW} relating g_s to irradiance (described as photosynthetic photon flux density, PPFD, $\mu\text{mol}/\text{m}^2/\text{s}$), temperature (T , degrees Celsius), vapour pressure deficit of the air (D , kPa) and soil water, respectively. f_{SW} can either be related to soil water potential (SWP in MPa) or plant available soil water expressed in volumetric terms (PAW in % vol/vol). f_{min} is the minimum daylight g_s under field conditions, expressed as a fraction of g_{max} .

¹ AOT40 = Ozone concentrations accumulated over a threshold of 40 ppb.

2.2. Estimating g_s for ‘real’ species modelling

The parameters and functions used for the estimation of ‘real’ species g_s are those defined in the mapping manual of the Convention on Long-Range Transboundary Air Pollution (LRTAP) (LRTAP Convention, 2010) with the exception of i. g_{max} values which have been revised according to newly available datasets since the publication of Karlsson et al. (2007) (i.e. LRTAP Convention, 2010; Marzuoli et al., 2009) and a recently modified diffusivity ratio of O_3 to water vapour of 0.663 (Grnhage et al., 2012); ii. a phenology function which is defined either by latitude according to LRTAP Convention (2010) or by information provided by holders of the experimental data included in this study (see Section 2.5 and Table 1 and Table S1 for further details) and; iii. the parameterisation for poplar, which was taken from Marzuoli et al. (2009) since a parameterisation for this species was previously not defined. All parameterisations used here are described in Table 1.

2.3. Estimating g_s using a simplified parameterisation

A second set of model runs was based on a simplified parameterisation of the model to test the feasibility of using less parameter-intensive estimates of environmental influences on g_s for tree species and groups of species. This simple model kept the same f_{SW} and f_{phen} formulation and parameterisation as described in Table 1. However, the formulations for f_{PPFD} , f_T and f_D have been revised with the intention to develop standard functions that can be used for all tree species. This simplified formulation of the flux model represents a new development of the ‘generic’ tree modelling concept presented in LRTAP Convention (2010), and

Table 2

Temperature parameterisation for all sites, irrespective of species that were exposed to O_3 there. For detailed information on sites, see Table 3.

Site	T_{min} (�C)	T_{opt} (�C)	T_{max} (�C)
Birmensdorf	9	21	45
Kuopio	3	16	45
��stad	1	17	45
Sch��nenbuch	7	20	45
Zugerberg	5	18	45
Col du Donon	–1	13	45
Ebro delta	12	25	45
Headley	6	20	45
Curno	10	24	45
Grignon	7	17	45

includes for the first time flux based DRR. These functions, described by a single formulation, are detailed in the following equations.

$$f_{PPFD} = f_{min} + 0.9 \cdot (1 - \text{EXP}(\text{light}_a \cdot \text{PPFD})) \quad (2)$$

where f_{PPFD} follows a rectangular hyperbola and light_a is 0.006; the structure of the formula is such that f_{min} also operates during the night-time period. This parameterisation has proved reasonable for the two most common species in the present dataset, birch (Uddling et al., 2004, 2005) and Norway spruce (Tarvainen et al., 2013), and the assumption is made that other species will follow a similar f_{PPFD} .

$$f_D = \min(1, 1 - 0.6 \cdot \ln(D)) \quad (3)$$

Table 1

Parameterisation of DO₃SE model for forest trees.

Parameter	Units	Beech	Birch	Temperate oak	Norway spruce	Norway spruce	Holm oak	Aleppo pine	Scots pine	Poplar
Region ^f		CCE	NE	ACE	NE	CCE	ME	ME	ACE	^c
g_{max}	mmol O_3 m ^{–2} PLA s ^{–1}	150	240	255	125	130	195	230	190	575/540
f_{min}	fraction	0.13	0.1	0.06	0.1	0.16	0.02	0.15	0.1	0.1
SGS ^e	year day	LF or DP	LF	LF or DP	DP	DP	1–365	1–365	DP	DP
EGS ^e	year day	LF or DP	LF	LF or DP	DP	DP	1–365	1–365	DP	DP
$f_{phen_lim}^a$	year day	–	–	–	–	–	80	80	–	–
$f_{phen_lim}^b$	year day	–	–	–	–	–	320	320	–	–
f_{phen_a}	fraction	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.8	0.0
f_{phen_b}	fraction	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)	1.0	1.0	(1.0)	(1.0)
f_{phen_c}	fraction	1.0	1.0	1.0	1.0	1.0	0.3	0.1	1.0	1.0
f_{phen_d}	fraction	(1.0)	(1.0)	(1.0)	(1.0)	(1.0)	1.0	1.0	(1.0)	(1.0)
f_{phen_e}	fraction	0.4	0.0	0.0	0.0	0.0	1.0	1.0	0.8	0.0
f_{phen_f}	days	20	20	20	20	–	130	130	40	20
f_{phen_g}	days	20	30	30	30	–	60	60	40	20
light_a		0.006	0.0042	0.003	0.006	0.01	0.012	0.013	0.006	0.006
T_{min}	�C	5	5	0 ^b	0	0	1	10	0	11
T_{opt}	�C	16	20	20 ^b	20	14	23	27	20	27
T_{max}	�C	33	200 ^a	35 ^b	200 ^a	35	39	38	36	36
VPD _{max}	kPa	1.0	0.5	1.0 ^b	0.8	0.5	2.2	1.0	0.6	2.1
VPD _{min}	kPa	3.1	2.7	3.25 ^b	2.8	3.0	4.0	3.2	2.8	3.7
SWP/PAW	MPa/% vol vol ^{–1}	If SWP, distinction between boreal/temperate and Mediterranean forest tree species; details of SWP and PAW parameterisation in footnote ^d								
Leaf dimension	cm	7.0	5.0	5.0	0.8	0.8	5.5	0.8	0.8	10

^a The T_{max} value is set at 200 C to simulate the weak response to high temperatures of Norway spruce and birch trees growing under Northern European conditions (the stomatal response is instead mediated by high VPD values). Hence, the T_{max} value should be viewed as a forcing rather than descriptive parameter.

^b ‘Generic’ deciduous parameterisation used as surrogate.

^c Parameterisation as given by Marzuoli et al. (2009) for young poplar trees, apart from g_{max} , which is based on site-specific measurements (575 mmol O_3 m^{–2} PLA s^{–1} = Curno, 540 mmol O_3 m^{–2} PLA s^{–1} = Grignon; italics represent values taken from beech ACE parameterisation).

^d boreal/temperate $f_{SWP} = \min\{1, \max\{f_{min}, 0.355 \cdot (-LWP_{pd})^{-0.706}\}\}$; Mediterranean $f_{SWP} = \min\{1, \max\{f_{min}, 0.619 \cdot (-LWP_{pd})^{-1.024}\}\}$, where LWP_{pd} is the predawn leaf water potential (MPa); $f_{PAW} = \min\{1, \max\{f_{min}, f_{min} + (1 - f_{min}) \cdot ((100 \text{ PAW}/FC) - \text{PAW}_{min})/(\text{PAW}_{crit} - \text{PAW}_{min})\}\}$, where FC is the field capacity, PAW_{min} is the soil texture dependent soil water content at minimum soil water potential and PAW_{crit} is 50% of PAW. For details see Bker et al. (2012).

^e LF = Latitude function according to LRTAP Convention (2010); DP = Data provided by data holders of experiments presented in Table 3. See also Table S1.

^f NE = Northern Europe; ACE = Atlantic Central Europe; CCE = Continental Central Europe; ME = Mediterranean Europe.

This f_D (except for its “min” formulation) is supported by the theory on tree hydraulics and has been shown to hold for a broad range of tree species (Oren et al., 1999). The commonly used bell-shaped f_T (LRTAP Convention, 2010) is given by:

$$f_T = \max\left(f_{\min}, \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}}\right) * \left(\frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}}\right)^{bt}\right) \quad (4)$$

where $T_{\min}$, T_{opt} and $T_{\max}$ are the species-specific minimum, optimum and maximum T , respectively. bt is defined as:

$$bt = (T_{\max} - T_{\text{opt}}) / (T_{\text{opt}} - T_{\min}) \quad (5)$$

Eq. (4) was only applied, if $T_{\min} < T \leq T_{\text{opt}}$. If $T < T_{\min}$,

$$f_T = f_{\min} \quad (6)$$

While traditionally f_T drops to 0 if $T_{\max} > T > T_{\text{opt}}$, here a non-limiting T effect on g_s is assumed if $T_{\max} > T > T_{\text{opt}}$, i.e. the effect of T on stomatal closure within this T range is thus assumed to be mediated through the effect of T on D : If $T_{\max} > T > T_{\text{opt}}$

$$f_T = 1 \quad (7)$$

and if $T > T_{\max}$, then

$$f_T = 0 \quad (8)$$

The f_T parameterisation was defined by analysing the site-specific growing season 24-h T at each site and using the 1st percentile and mean temperatures as the $T_{\min}$ and T_{opt} values respectively. The growth period was defined as for the ‘real’ species parameterisation (Table 1 and Table S1). Hence, this parameterisation is site- rather than species-specific. $T_{\max}$ was fixed at 45 °C which is an average maximum T below which the most drought-adapted European trees can maintain their physiological activity (Larcher, 2003). The simplified parameterisation of f_T for all sites is also described in Table 2. Finally, $f_{\min}$ was set to 0.1 for the simplified parameterisation.

2.4. Estimating PODy and AOT40

The calculation of PODy requires estimates of stomatal O_3 flux (F_{st}) which are made according to the LRTAP Convention (2010). F_{st} ($\text{nmol } O_3 \text{ m}^{-2} \text{ Projected Leaf Area (PLA) s}^{-1}$) is calculated according to Eq. (9) which accounts for deposition to the cuticle through incorporation of the leaf surface resistance (r_c) and boundary layer resistance (r_b) terms:

$$F_{st} = c(z_1) * g_s * \frac{r_c}{r_b + r_c} \quad (9)$$

where $c(z_1)$ is the concentration of O_3 at the top of the canopy (nmol m^{-3}) at height z_1 (m), g_s is in m s^{-1} , r_b is the leaf quasi-laminar resistance and r_c the leaf surface resistance, both given in s m^{-1} . For further details on the resistance scheme see LRTAP Convention (2010). The accumulated F_{st} above an O_3 stomatal flux threshold of y ($\text{nmol m}^{-2} \text{ s}^{-1}$) provides the PODy index and is calculated according to Eq. (10).

$$\text{POD}_y = \sum_{i=1}^n \max [F_{st_i} - y, 0] * \Delta t \quad (10)$$

where F_{st_i} is the stomatal O_3 flux ($\text{nmol } O_3 \text{ m}^{-2} \text{ PLA h}^{-1}$) and y is the threshold stomatal flux per projected leaf area (PLA) (in $\text{nmol } O_3 \text{ m}^{-2} \text{ s}^{-1}$). The calculation of PODy is based on hourly values (F_{st_i}),

so $\Delta t = 1 \text{ h}$. n is the number of hours within the defined growth period. We trialled small y increments of 0.1 from 0 to 6 $\text{nmol } O_3 \text{ m}^{-2} \text{ PLA s}^{-1}$ to facilitate a more precise determination of the ‘ y ’ threshold.

Estimates of AOT40 (Accumulated Over a Threshold of 40 ppb ozone) are performed using the methodology outlined in the LRTAP Convention (2010):

$$\text{AOT40} = \sum_{i=1}^n \max [c_i - 40, 0] * \Delta t, \quad (11)$$

Where 40 is the threshold concentration (in ppb) and the calculation of AOT40 is based on hourly values (c_i) so that $\Delta t = 1 \text{ h}$ and n is again the number of hours within the defined growth period.

The model runs were performed using the interfaced version of DO3SE (<http://www.sei-international.org/do3se>). This code has been rigorously tested and provides a definitive set of algorithms that represent the current formulations and parameterisations to derive PODy (LRTAP Convention, 2010) as well as AOT40 values.

2.5. Description of sites and datasets

This study used O_3 experimental fumigation or filtration datasets collected for eight different species: birch (*Betula pendula*), beech (*Fagus sylvatica*), temperate oak (*Quercus robur* or *petraea*), Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), Aleppo pine (*Pinus halepensis*), Holm oak (*Quercus ilex*) and poplar hybrids (*Populus maximowicz* $\times$ *Populus berolinensis* or *Populus deltoides* $\times$ *Populus nigra*). These datasets were from ten different sites in seven countries (Sweden, Finland, U.K., France, Switzerland, Spain and Italy), thereby representing all major European climatic zones defined in the UNECE Mapping Manual (LRTAP Convention, 2010). Six experiments from France, Italy, Spain and Sweden contained reduced water supply treatments created either by i. reduced irrigation for up to 10 weeks, ii. reduced precipitation (with the help of an Open Top Chamber (OTC) roof) for up to 20 weeks or iii. no irrigation but full precipitation for the entire growing season. Further details of these treatments by dataset are provided in Table 3.

One of the datasets that had been included in the earlier study by Karlsson et al. (2007) is excluded from this study due to too few replicates having been conducted in the experiment. This study also includes three new datasets for Holm oak and poplar collected from sites in France, Italy and Spain (Table 3).

Apart from two open-field systems in Grignon (Le Thiec, pers. comm.; Ahmad et al., 2012) and Kuopio (Oksanen, 2003), all other sites were equipped with Open Top Chambers (OTC) for the exposure of young trees to O_3 . Details about the individual experiments can be found in Table 3 and the papers referenced therein. The control treatment in these experiments was either charcoal-filtered or ambient (non-filtered) air, and the O_3 treatments were ambient air, charcoal filtered plus additional O_3 or ambient air plus additional O_3 . The response parameter used for the derivation of the DRR was wherever possible total biomass; where root biomass was not available, above-ground biomass was used.

In LRTAP Convention (2010), the growing season is estimated using the latitude model for forests that do not have a year-round growth habit. However, the experiments included in this analysis were predominantly performed in OTCs and the fumigation or filtration periods were sometimes outside of the latitude function-derived growth period. Hence, only experiments that captured 60% or more of this period were included here to ensure that O_3 exposures covered the majority of the estimated growing season. Table S1 gives the O_3 flux accumulation period, as defined by the

fumigation or filtration period.

The calculation of PODy required that the practitioner of each experimental site provided a full complement of hourly meteorological data from which *T*, *D* and *PPFD* could be determined according to standard methods (Jones, 1992). Hourly canopy height *O*₃ concentration data as well as a seasonal profile of soil water status was also required.

2.6. Derivation of *O*₃ flux based dose–response relationships

The PODy and AOT40 were accumulated over the entire length of each individual growing season of each experiment. When experiments covered multiple growing seasons, the PODy and AOT40 of the fumigation periods of all growing seasons were summed and then divided by the number of growing seasons to obtain the mean annual PODy and AOT40.

In order to standardise the biomass response data from experiments of differing duration, the following formula (Fhrer, 1994) was applied to calculate the mean annual percentage reduction in biomass relative to the control (*RB*_{annual}):

$$RB_{\text{annual}} = 100 * (B_{\text{treat}}/B_{\text{control}})^{(1/n)} \quad (12)$$

where *B*_{treat} and *B*_{control} represent the biomass at ambient or elevated *O*₃ exposures and a control at zero AOT40 or PODy respectively, and *n* is the number of growing seasons. This equation assumes a constant ozone-induced percentage reduction in biomass accumulation across years in multi-year experiments.

For experiments that used non-filtered ambient air (NF) rather than CF as control treatment or that had a PODy or AOT40 of greater than 0 for the CF treatment, the biomass of the control was recalculated as the Y-intercept value resulting from the linear regression of biomass (Y-axis) and *O*₃ exposure (X-axis) data (Fhrer, 1994). The reduction in biomass for each treatment was calculated relative to the hypothetical biomass at zero AOT40 or zero PODy, which enabled the comparison of experiments that had either CF or NF control treatments. Species were investigated either as single or

groups of species. Broader species groupings allowed analysis of responses according to plant functional types, with needleleaf trees (*Picea abies*, *Pinus halepensis* and *Pinus sylvestris*) and broadleaf deciduous trees (*Betula pendula*, *Fagus sylvatica*, *Populus spec.*, *Quercus robur* and *Quercus petraea*) being used as comparative groupings.

For the derivation of concentration- and flux-based DRR, the relationships between *RB*_{annual} and the mean annual AOT40 or PODy were fitted using the linear regression and mixed linear regression in R (version 3.1.3) and covering data from all *O*₃ treatments including the control, without forcing the regression through 1 on the Y-axis. Each data point included in the regression analysis represented a single value for each species, treatment and harvest of each experiment. The *R*² and confidence intervals (C.I.) of the slope of the regression line were used to test the robustness of DRR.

For the linear regression and mixed linear regression the functions *glm* and *lme* were used, respectively (package *nlme*; Pinheiro et al., 2015). A correction for heteroscedasticity was made using the option *weights = varPower()*. The Akaike Information Criteria (AIC) was used to decide which regression type was appropriate. In most cases the simple regression with heteroscedasticity correction yielded the lowest AIC (and hence the strongest regression), except for the groups “needleleaf trees” and “*Quercus ilex* and *P. halepensis*”, where the inclusion of species as cluster improved the AIC. The ‘y’ thresholds of the PODy indices that yield intercept C.I. that include 100% can be deemed to provide statistically robust DRR.

However, it is also useful to set some objective criteria by which a clear indication of the relative performance of different ‘y’ (nmol m^{−2} s^{−1}) thresholds can be assessed when comparing between species and PFTs. This was achieved by analysing key attributes of the (mixed) linear regressions between PODy and biomass reduction, namely the Y-axis intercept and its C.I. and *R*² values of each dose–response regression with incrementally increasing ‘y’ thresholds. These attributes used two criteria: a) that the C.I. of the intercept includes 100% and b) that the *R*² value is within 2% of the maximum *R*² value.

Table 3

Description of experiments. Those shaded in grey are new datasets as compared to Karlsson et al. (2007). AA = ambient air; CF = Charcoal-filtered; NF = Non-filtered (ambient); CF/NF+/++ = Charcoal-filtered or Non-filtered plus addition of *O*₃. OTC = Open top chamber.

Species	Site	Exposure system	Control treatment	Ozone treatments	Experiment duration (yrs)	Red. H ₂ O treatment	Tree age at start of experiment (yrs)	Response parameter	Reference
<i>Betula pendula</i>	Birmensdorf (CH)	OTC	CF	CF+, CF++, CF+++	1	No	Cuttings	Total biomass	Uddling et al. (2004)
	Kuopio (FIN)	Open-field	AA	AA+	2, 2, 5	No	1	Perennial biomass	Oksanen (2003)
	��stad (S)	OTC	NF	NF+, NF++	1, 2	No	<1	Perennial biomass	Karlsson et al. (2003)
<i>Fagus sylvatica</i>	Sch��nenbuch (CH)	OTC	CF	NF	2	No	<1	Total biomass	Braun and Fl��ckiger (1995)
	Zugerberg (CH)	OTC	CF	NF	2, 3	No	<1	Total biomass	Braun and Fl��ckiger (1995)
<i>Picea abies</i>	��stad (S)	OTC	CF	NF, NF+	1, 2, 3, 4	Yes	3	Total biomass	Ottosson et al. (2003), Karlsson et al. (2004)
	Sch��nenbuch (CH)	OTC	CF	NF	2	No	<1	Total biomass	Braun and Fl��ckiger (1995)
	Zugerberg (CH)	OTC	CF	NF	1, 2, 3	No	<1	Total biomass	Braun and Fl��ckiger (1995)
<i>Pinus halepensis</i>	Ebro Delta (SP)	OTC	CF	NF, NF+	3	Yes	2	Total biomass	Alonso et al. (2003), Elvira et al. (2007)
<i>Pinus sylvestris</i>	Headley (U.K.)	OTC	NF	NF+	2	No	1	Above-ground biomass	Medlyn et al. (1999)
<i>Populus spec.</i> ^a	Curno (I)	OTC	CF	NF	1	Yes	1–2	Above-ground biomass	Marzuoli et al. (2009), Pollastrini et al. (2010)
	Grignon (F)	Open-field	NF	NF+, NF++	1	No	≤1	Above-ground biomass	Le Thiec, pers. comm., Ahmad et al. (2012)
<i>Quercus ilex</i>	Ebro Delta (SP)	OTC	CF	NF, NF+	2	Yes	2	Above-ground biomass	Alonso et al. (2014)
<i>Quercus robur</i>	Col-du-Donon (F)	OTC	CF	NF, NF+	2	Yes	2	Total biomass	Dixon et al. (1998)
<i>or petraea</i>	Headley (U.K.)	OTC	NF	NF+	2	No	1	Above-ground biomass	Medlyn et al. (1999)

^a Either *Populus maximowicz* × *Populus berolinensis* (Curno) or *Populus deltoides* × *Populus nigra* (Grignon).

Fig. 1. Intercept and R^2 for various O_3 flux thresholds (y) of each species or species group. The black bars represent the y range where the confidence interval of the intercept includes 100% and where the R^2 is within 2% of the maximum R^2 .

Table 4
Overview of 'y'-range for species and species-groups according to criteria defined in Section 2.6.

Species/Species-group	'y'-range
<i>Picea abies</i>	0.2–1.2
<i>Fagus sylvatica</i> & <i>Betula pendula</i>	1.6–5.1
<i>Quercus ilex</i> & <i>Pinus halepensis</i>	0.0–0.7
Broadleaf deciduous trees	1.3–3.8
Needleleaf trees	0.9–2.5

3. Results

3.1. Derivation of y threshold

The y range according to the criteria outlined in Section 2.6 is largest for beech/birch and broadleaf deciduous trees, and smallest for Holm oak and Aleppo pine (black bars in Fig. 1; Table 4). The confidence intervals of the intercept were smaller for needleleaf as compared of broadleaf trees. The maximum R^2 was highest for Holm oak and Aleppo pine and smallest for Norway spruce (Fig. 1;

Fig. 2. Dose–response relationships for *Picea abies* for O₃ indices A) AOT40, B) standard “real species” model POD₁ and C) simplified model POD₁.

Fig. 3. Dose–response relationships for *Fagus sylvatica* and *Betula pendula* for O₃ indices A) AOT40, B) standard “real species” model POD₃ and C) simple model POD₃.

NB: The displacement of the intercept (and its C.I.) of Norway spruce is due to one value dominating the intercept above $y = 4.7 \text{ nmol m}^{-2} \text{ s}^{-1}$.

For the following definition of DRR, exemplary 'y' thresholds (rounded to the next natural number) closest to the maximum R^2 of each species and species group were chosen.

3.2. Dose–response relationships

For *Picea abies* (Fig. 2), the flux based DRR using the simple model is on a par with the AOT40 based DRR ($R^2 = 0.37$ and 0.38 ; intercept = 1.0 and 1.0 ; slope = -0.0019 and -0.0024 , respectively; for standard errors (S.E.) and confidence intervals (C.I.), see Tables S2 and S7), while the flux based DRR using the standard “real-species” model is slightly less strong ($R^2 = 0.34$; intercept = 1.0 ; slope = -0.0022 ; for S.E. and C.I., see Table S2).

The DRR for *Betula pendula* and *Fagus sylvatica* (Fig. 3) based on the flux based index POD_3 with both g_s model parameterisations ($R^2 = 0.70$ and 0.68 ; intercept = 0.99 and 1.00 ; slope = -0.0137 and -0.0097 for the “real-species” and simple model, respectively; for S.E. and C.I., see Table S3) and the concentration-based index AOT40 ($R^2 = 0.68$; intercept = 0.99 ; slope = -0.0076 ; for S.E. and C.I., see Table S7) perform equally well.

For the Mediterranean evergreen species *Quercus ilex* and *Pinus halepensis* (Fig. 4), both the POD_1 response relationships using the “real species” and simple model ($R^2 = 0.72$ and 0.63 ; intercept = 1.01 and 1.01 ; slope = -0.0024 and -0.0037 , respectively; for S.E. and C.I., see Table S4) clearly outperform the AOT40-based DRR ($R^2 = 0.42$; intercept = 1.00 ; slope = -0.0005 ; for S.E. and C.I., see Table S7).

Fig. 5 shows the stronger flux-based DRR ($R^2 = 0.52$ and 0.45 ; intercept = 1.00 and 1.00 ; slope = -0.0042 and -0.0038 for the “real species” and simple model, respectively; for S.E. and C.I., see Table S5) as compared to the AOT40-based DRR ($R^2 = 0.38$; intercept = 1.00 ; slope = -0.0021 ; for S.E. and C.I., see Table S7) for three needleleaf tree species. The performance of all needleleaf tree DRR is better than the separate respective DRR for *Picea abies* alone (Fig. 2).

Fig. 6 compares the POD_3 -response relationships for five broadleaf deciduous tree species using the “real-species” and simple model with the AOT40-response relationship. While the differences in the R^2 are fairly small (0.41 , 0.38 and 0.34 for AOT40, “real species” and simple model, respectively), the slopes differ much more (-0.0061 , -0.0098 , -0.0082 for AOT40, “real species” and simple model, respectively; for S.E. and C.I., see Tables S6 and S7). The performance of all broadleaf deciduous tree DRR is worse than the separate respective DRR for the two broadleaf species *B. pendula* and *Fagus sylvatica* (Fig. 3).

The Tables S2 to S7 provide an overview of all AOT40- and flux-based DRR.

3.3. Effect of reduced water supply

Datasets of six experiments using five different species (*Picea abies*, *P. halepensis*, *Quercus robur*, *Q. ilex* and *Populus spec.*) comprised data points representing both well-watered and reduced-water conditions. The data points of the reduced water supply treatments are positioned within the general data cloud of Figs. 2 and 4–6 (not indicated in these figures), which suggests that they do not represent clearly distinguishable O_3 -uptake or

Fig. 4. Dose–response relationships for Mediterranean evergreen species *Quercus ilex* and *Pinus halepensis* for O_3 indices A) AOT40, B) standard “real species” model POD_1 and C) simple model POD_1 .

Fig. 5. Dose–response relationships for needleleaf tree species for A) AOT40, B) standard “real species model” POD₂ and C) simple model POD₂.

Fig. 6. Dose–response relationships for broadleaf deciduous tree species for A) AOT40, B) standard “real species model” POD₃ and C) simplified parameterisation POD₃.

-response conditions as compared to the well-watered treatments.

To more clearly observe the size of the effect of reduced water supply on g_s and consequently on PODy, the experimental datasets that included a water stress treatment (15 data points in total) were directly compared with the well-water treatments of the same experiments using the “real species” model. These two sets of PODy data were plotted against each other so that the deviation from the 1:1 line indicated the effect of water stress on PODy (Fig. 7).

There was a significant (at 95% confidence level) but small (9%) deviation from the 1:1 line, suggesting that the effect of reduced water availability on g_s and hence O_3 flux was small in the experiments included in this study.

4. Discussion

We analysed data from 24 European field experiments to derive O_3 flux based DRR for Norway spruce, beech and birch, Holm oak and Aleppo pine, as well as the two PFTs of broadleaf deciduous trees and needleleaf trees.

The inclusion of Holm oak and Aleppo pine datasets for the first time enabled the derivation of a combined DRR for Mediterranean trees only (a separate DRR for Holm oak was recently published by Alonso et al. (2014)). Despite these two species representing different PFTs, their grouping is justified due to both of them being evergreen, physiologically active throughout the year and able to cope well with Mediterranean climatic conditions, including drought. However, it should be noted that broadleaf deciduous species are also common in the Mediterranean and that these species have been shown to be more sensitive to O_3 than the broadleaf evergreen species Holm oak used in this study (Calatayud et al., 2011).

Rather than suggesting species- or PFT-specific “suitable” ‘y’ thresholds, we give a range of different species- or PFT-specific ‘y’ thresholds according to statistical criteria outlined in Section 2.6. Firstly, this study shows that all of the ‘y’ thresholds (from 0 to 6) provide statistically robust DRR for all species and PFTs when considering the intercept CIs, with the exception of ‘y’ thresholds

above $4.7 \text{ nmol m}^{-2} \text{ s}^{-1}$ for needleleaf trees. This study therefore supports the current ‘y’ thresholds used by LRTAP Convention (2010). When considering the intercept and R^2 statistics in combination (to aid the objective comparison of these key statistical characteristics between species and PFTs), the study suggests that needleleaf trees may have a lower ‘y’ as compared to broadleaf deciduous trees, which might be an indication that the latter have a higher detoxification potential. In fact, Temmerman et al. (2002), Musselman et al. (2006) and Dizengremel et al. (2009) discuss the concept of varying detoxification potentials of trees in detail. They agree that given the biochemical processes (i.e. antioxidant capacity, turnover and transport) that are known to be involved in ozone scavenging, it is certain that plants have a capacity for detoxification and that this varies between species. However, the complexity of these biochemical processes currently makes it difficult to quantify this capacity either absolutely or in relative terms for different species or species groups.

The new flux-based DRR for Norway spruce is associated with a lower R^2 (0.34) than reported by LRTAP Convention (2010) ($R^2 = 0.55$), mainly because one dataset that had been used in these two previous studies and that was characterised by high PODy values had to be excluded due to a limited replication of experimental treatments. In addition, a new parameterisation for g_{max} and changes in the start and end of the phenological period (these changes applied to all species involved in this study) have also caused differences by increasing the maximum stomatal O_3 flux and changing its accumulation period.

In contrast, the new DRR for beech/birch with R^2 values of 0.70 is an improvement over DRR reported previously ($R^2 = 0.64$ in LRTAP Convention, 2010). This result is also supported by a recent epidemiological study (Braun et al., 2014) that also emphasised the transferability of DRR derived from young trees to mature trees.

The grouping of species into PFTs of broadleaf deciduous and needleleaf trees has led to fairly robust DRR; while the DRR for broadleaf deciduous trees was less robust than for beech and birch, the DRR for needleleaf trees was actually more robust than for the Norway spruce. The new DRR for PFTs clearly and uniformly demonstrate substantial loss in biomass with increasing O_3 flux for European trees. As such, these new DRR for PFTs (plus that for Mediterranean trees) will expand the applicability of the flux based risk assessment method.

It would be desirable to use these new DRR to describe the relative sensitivities of different species and species groups. Ordinarily, this would be described by the slope of the regressions (provided for each of the ‘y’ threshold increments in the supplementary material in Table S2 to S6). However, this is complicated by the fact that the slope of the regression will also depend upon the ‘y’ threshold, with higher ‘y’ thresholds tending to have steeper slopes (as the index accumulates less quickly). Therefore it is not so straightforward to compare species sensitivities based on slopes alone. However, if we were to assume that all species had a ‘y’ threshold of 1 as suggested by LRTAP Convention (2010), we would get the following ranking in terms of sensitivity of the species groups and plant functional types (from higher to lower sensitivity): Beech/birch > Broadleaf deciduous trees > Needleleaf trees > Holm oak/Aleppo pine > Norway spruce.

For application on a broader-scale, we tested the concept of using a simplified parameterisation to estimate PODy, by comparing flux–effect relationships derived using the “real species” parameterisation with those that were derived using the simplified parameterisation. These two approaches differed in terms of the parameterisation of their PPFD, T and D functions. Based on the statistical analysis of the DRR (see Tables S2 to S6), the model using the simplified parameterisation slightly outperformed the “real species” model for Norway spruce, whereas for all other

Fig. 7. Regression for PODy of well-watered vs. reduced-water treatments for five experiments representing the following trees species: *Picea abies*, *Pinus halepensis*, *Quercus robur*, *Quercus ilex* and *Populus spec.* The 1:1 line (dashed) is also shown. N.B. Reduced-water represents instances when g_s was limited by soil water availability according to the DO_3SE model (Büker et al. (2012)).

species or PFT the opposite was the case (especially for the Mediterranean tree species). These results indicate that for Norway spruce, beech/birch, broadleaf deciduous trees and – to a lesser extent – needleleaf trees the differences in the performance between both models is not so severe that the application of the simple model would introduce a major loss in accuracy in predicting O_3 fluxes. However, the same is not the case when applying the simplified parameterisation for Mediterranean tree species, where presumably significant uncertainties would be introduced as compared to the “real species” model, thereby questioning the general suitability of the simplified parameterisation of the model. As such, the less parameter-intensive use of the simplified parameterisation for large scale (e.g. pan-European) risk assessments is not yet possible. It would be useful to further assess whether the uncertainty introduced by applying the simplified parameterisation would vary significantly between European climatic zones and tree species.

The inclusion of datasets representing experiments performed under restricted water availability neither substantially improved nor worsened the DRR; the data points from restricted water experiments fell within the range of data points from well-watered experiments. This provides further support for use of flux based methods as compared to the concentration-based AOT40 approach, which cannot account for environmental impacts on O_3 uptake by plants. The difference in the performance between the flux based and the AOT40-based DRR found in this study was comparable to earlier studies by Karlsson et al. (2007) and the LRTAP Convention (2010), despite the fact that the DO₃SE model now has a much more sophisticated soil moisture deficit module that has been successfully validated (Büker et al., 2012). Further analysis found that the reduced water treatments applied in the various experiments did lead to significant but small reductions in modelled g_s , hence leading only to slight reductions in O_3 flux (Fig. 7), which is in contrast to earlier – mostly Mediterranean – studies, for instance by Alonso et al. (2014), who used a local parameterisation of the flux model. The periods of reduced water availability were too short and moderate to represent severe drought conditions. However, actual Mediterranean conditions are likely to incur far greater soil water stress over longer time periods than were applied in the experimental studies investigated in this analysis, with consequent limitations on g_s and hence reduced POD_y. Therefore it is imperative to include the fSW function in models used to estimate POD_y values. More prolonged episodes of reduced water availability would have had to be applied to significantly reduce g_s and hence O_3 fluxes, which could have led to an even more pronounced difference in the performance of the AOT40 as compared to the flux based risk assessment approach.

5. Conclusions

The analysis presented here derived new O_3 flux based DRR for nine European forest tree species using varying statistically derived flux thresholds according to the species sensitivity to O_3 . Flux based DRR were mostly more robust than concentration based DRR. The study showed that it is possible to define DRR both by species as well as by plant functional types and that the use of a simplified parameterisation of the g_s model can provide reasonable accuracy in the calculation of POD_y for non-Mediterranean tree species. The findings suggest that critical levels as published by the UNECE (LRTAP Convention, 2010) could be re-defined using the updated POD_y estimates and biomass response datasets presented here. They also support the ‘y’ thresholds currently used in the POD_y metrics but do suggest that there may be justification in considering differential ‘y’ thresholds for broadleaf and needleleaf species (the former having a higher ‘y’ threshold than the latter) based on

an objective characterisation of key statistical parameters and how these compare between species and PFT groupings.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2015.06.033>.

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