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Title: Plant Species Sensitivity Distributions for ozone exposure

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ABSTRACT

This study derived Species Sensitivity Distributions (SSD), representing a cumulative stressor-response distribution based on single-species sensitivity data, for ozone exposure on natural vegetation. SSDs were constructed for three species groups, i.e. trees, annual grassland and perennial grassland species, using species-specific exposure-response data. The SSDs were applied in two ways. First, critical levels were calculated for each species group and compared to current critical levels for ozone exposure. Second, spatially explicit estimates of the potentially affected fraction of plant species in Northwestern Europe were calculated, based on ambient ozone concentrations. We found that the SSD-based critical levels were lower than for the current critical levels for ozone exposure, with conventional critical levels for ozone relating to 8-20% affected plant species. Our study shows that the SSD concept can be successfully applied to both derive critical ozone levels and estimate the potentially affected species fraction of plant communities along specific ozone gradients.

Capsule: Species Sensitivity Distributions offer opportunities in ozone risk assessment to both derive critical levels and estimate the affected fraction of a plant community.

Key words: *Ozone; Ecological Risk Assessment; AOT40; Species Sensitivity Distribution; Potentially Affected Fraction*

INTRODUCTION

Northern Hemisphere tropospheric background ozone concentrations have increased over recent decades, as peak concentrations have fallen in North America and Europe (Derwent et al. 2007; Vingarzan, 2004). Background concentrations are predicted to further increase with 0.5 – 2% per year over the next 50 years primarily due to elevated emissions of nitrogen oxides and volatile organic compounds (Emberson et al., 2003; Royal Society, 2008). The adverse effects of ozone pollution on plants, including trees and grassland species, are of considerable concern (Emberson et al. 2007; Mills et al., 2007a, b). Some of these effects include growth and seed production reduction (Booker et al., 2009), premature senescence (Tonneijck et al., 2004), reduced ability to withstand stressors (Wilkinson and Davies, 2009), and an increase in leaf injury (Manning et al., 2002).

Critical levels are based on relationships between ozone concentrations and effects such as yield loss and biomass reduction (Hayes et al., 2006; Pleijel et al., 2007; Tuovinen et al., 2007). These levels are expressed as an Accumulated exposure Over a Threshold of 40 ppb (AOT40) and are based on sensitive but ecological relevant species (LRTAP, 2010, Matyssek et al. 2007). These species, and corresponding critical levels, are used as indicators to determine the risk for species groups or plant communities (Musselman and Lefohn, 2007). For example, critical levels of *Trifolium sp*. are assumed representative for all species of the productive grassland community (Klingberg et al., 2011). For monoculture arable crops and productive trees, such an approach of defining a critical level based on a single species for that community is possible. However, for semi-natural plant communities, with the large range of species present, an approach based on a single indicator such as *Trifolium* ignores the wide range of sensitivity across all the component species (Hayes et al., 2007; Mills et al. 2007b). To date, an approach which gives the affected fraction of a species assemblage due to ozone exposure is lacking in risk assessment for semi-natural vegetation (Ashmore, 2005; Paoletti and Manning, 2007).

In contrast, in most areas of ecotoxicology, Species Sensitivity Distributions (SSDs) are used (1) to derive environmental quality objectives of chemicals set equal to the concentration at which 5% of the species are affected (HC_5), and (2) to estimate the fraction of species affected at different exposure concentrations of chemicals (Posthuma et al., 2002). An SSD is a cumulative distribution of responses of different biological species to the same stressor (Van Straalen et al., 1989). The SSD concept is a standard approach in ecotoxicology which is applicable to ozone risk assessment. It offers opportunities to both derive critical levels and estimate the affected fraction of species within a plant community along a specific ozone gradient.

The goal of this study was to develop SSDs for ozone exposure on natural vegetation. Our study includes 96 plant species. SSDs were constructed from species-specific ozone-response data provided by a comprehensive review of scientific literature and databases. Species were grouped according to response type (decrease or no decrease of biomass) and taxonomy (trees, annual and perennial grassland species). Critical threshold levels for ozone based on HC_5 were compared with AOT40-based critical levels commonly used in environmental policy assessment for ozone exposure. Finally, we show how the SSDs can be applied in practice by deriving spatially explicit estimates of potentially affected fraction of plant species in Northwestern Europe.

METHODS

In order to derive SSDs, we first gathered species-specific ozone exposure-response functions from the literature. In these functions the measure of ozone exposure was expressed as AOT40, calculated as the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb during daylight hours. The exposure-response functions were used to calculate for each species the AOT40 value related to a 10% effect (EC_{10}). These species-specific EC_{10} values were subsequently used to derive the average and standard deviation of the SSD for each vegetation type. The steps from gathering species-specific data on ozone effects and acquiring SSDs to deriving HC_5 values are described below.

Data gathering

Data on the effects of ozone concentrations on plants were collected from peer-reviewed studies published up to April 2012. The following keywords were used in the Boolean search (incl. keyword extensions) in Web of Science: (1) ozone; and (2) either vegetation, plant, tree, grassland; and (3) either critical levels, dose-response relationship, exposure, response, biomass; and (4) either open top chamber (OTC), AOT40, Free-Air Concentration Enrichment (FACE), exposure based model. This literature search provided 980 peer-reviewed studies to be considered. In addition to the Boolean search we used the data from the OZOVEG database (Hayes et al., 2007).

Data selection

Following Mills et al. (2007a) and Hayes et al. (2007), ozone exposure-response data from individual species were only included when the following criteria were met:

(1). It should not be a factorial experiment, testing for the effect of a treatment variable in addition to ozone, e.g. CO2 + O3 exposure, except when the specific effect of ozone without the treatment variable could be quantified.

(2) Experiments should be conducted under 'close to field' conditions, either using an open-top chamber (OTC), field release system (e.g. Eastburn, 2006) or solardome (e.g. Rafarel et al., 1995).

(3) The accumulated exposure above the critical 40ppb level should be at least be 21 days to ensure chronic exposure.

(4) The mean ozone concentration for any hour of the day should be maximum 100 ppb to take only realistic field conditions into account.

(5) Only ozone response data for individual species and not higher taxonomic groups (e.g. family, class, etc.) were considered. An exception was made for genus-level records in case no other species belonging to that particular genus was listed.

(6) Experiments should report the change in biomass. This endpoint is commonly used for ozone risk assessment in plants (LRTAP, 2010).

Ozone exposure-response relationships were found for a total of 96 species. For grassland species functions available from the OZOVEG database, along with new data for the additional species were used (Hayes et al., 2007), for trees data presented in Calatayud et al. (2011), Karlsson et al. (2003), Karlsson et al. (2004), Landolt et al. (2000), Skärby et al. (2004) was used.

Data handling

First, species synonyms were excluded using The Plant List (2010) to avoid double counting of species names. The effects of ozone on biomass were calculated relative to the charcoal-filtered air treatment (or occasionally non-filtered air if no charcoal filtered control was used). EC_{10} values were then calculated using the standardized dose-response functions. Species exhibited two types of response when exposed to ozone, either biomass reduction (negative slope) or no biomass decrease (positive slope). The linear functions for biomass decrease were converted as follows:

$$EC_{10} = \frac{-0.1 \cdot b}{a} \tag{1}$$

,where b is the intercept and a is the slope of the linear function.

A list of all species with their dose-response functions and EC_{10} values can be found in the Supplementary information (S1, S2 and S3).

Species sensitivity distributions

Species Sensitivity Distributions (SSDs) were developed for three separate groups of species, i.e. trees, annual grassland species and perennial grassland species. For each group there were two effect definitions:

- one SSD was derived based on EC_{10} values for biomass reduction only;
- one SSD was derived for biomass reduction, corrected for the fraction of species with no biomass reduction (f_{nbd}).

SSDs were derived in the following way. First the EC_{10} data were log-transformed. Second, the mean (μ) and standard deviation (σ) of the log EC_{10}-data were calculated. Assuming a lognormal SSD for ozone exposure, the parameters μ and σ were then used to derive the Potentially Affected Fraction (PAF):

$$PAF = \frac{a}{\sigma \cdot \sqrt{2 \cdot \pi} \cdot AOT40 \cdot \ln 10} \cdot \int_0^{AOT40} \exp\left(-\frac{1}{2} \cdot \left(\frac{\log(AOT40) - \mu}{\sigma} \right)^2 \right) dAOT40 \tag{2}$$

, where a is 1 for the SSD derived based on EC_{10} values for biomass reduction only and a equals 1- f_{nbd} for the SSD derived including the fraction of species with no biomass reduction. AOT40 represents the ambient ozone exposure.

Differences in sensitivity between the species groups were investigated by comparing the means (μ) and variances (σ). The log10-transformed EC_{10} values were tested for normality with the Kolmogorov Smirnov test. The means were compared with the Independent t-test and the variances (σ) were compared using the Levene's test. All tests were executed with SPSS 17.0 for Windows.

Critical levels

Hazardous exposure concentrations for which 5% of the species assemblage remains unprotected (HC_5) were derived for each species groups and their respective response types. The HC_5 for the species with biomass reduction only was calculated following the procedure described by Aldenberg and Jaworska (2000):

$$LogHC_5 = \mu - k \cdot \sigma \tag{3}$$

where k is the extrapolation constant for 95% species protection. Aldenberg and Jaworska (2000) present extrapolation constants for the estimation of the $\log(HC_5)$ based on the assumption of normal species sensitivity distributions for the log-transformed toxicity data. To assess the uncertainty of the HC_5 the 90% confidence interval was calculated following Aldenberg and Jaworska (2000).

The HC_5 for the species assemblage including the fraction of species with no biomass reduction was derived by calculating the concentration at which $5/(1-f_{nbd})$% of the sensitive species is affected.

PAF levels corresponding to the critical levels recommended by the LRTAP Convention (2010) were determined using the lognormal SSD function. The 90% confidence interval was calculated following methods adapted from Aldenberg and Jaworska (2000).

Impact assessment

Maps of the potentially affected fraction (PAF) of species were compiled to determine the impact of ozone exposure on annual and perennial grassland species in Northwestern Europe. A spatially explicit grid-based approach on a 0.5 x 0.5 degree (i.e. ca. 50km x 50km at 60º N) resolution was applied. Grid-specific AOT40 exposure concentrations for 2010 were obtained using the EMEP model (Jonson et al. 2001). The AOT40 values were based on a growing season of May-July at a height of 1m above the ground. In each grid the PAF was derived for each species groups using the AOT40 exposure values as input in the SSD (equation 3).

RESULTS

Species sensitivity distributions

Exposure-response functions were determined for 25 annual grassland species, 62 perennial grassland species, and 9 tree species. The full data set is given in the SI (tables S1, S2 and S3). The percentage of species in the dataset that exhibited a biomass reduction was 88% for annual grassland species, 63% for perennial grassland species and 100% for tree species. According to the Kolmogorov Smirnov test all EC_{10}-data were normally distributed.

Figure 1 shows the species sensitivity distributions for annual grassland species, perennial grassland species and trees based on EC_{10}-data (a) and with the fraction of species with no biomass decrease included (b). Significant differences in means were found for annual and perennial grassland species, i.e. $p = 0.01$ for biomass reduction. Significant differences in variances were found for annual grassland species and trees. All results of the statistical testing of differences in means and variances can be found in the SI (S4).

Figure 1

Critical levels

HC_5 values varied from 1.3 to 4.1 ppm.h for the various species groups and effect definitions with no statistically significant differences (Table 1). The HC_5 values for annual and perennial grassland species were consistently lower than the corresponding critical levels. The PAFs relating to the current critical levels were derived for each species group. These indicated that potentially 8% of tree species, 17% of perennial grassland species, and 20% of annual grassland species have a growth reduction of at least 10% due to ozone exposure at the current critical level.

Table 1

Impact assessment

The actual PAF of grassland species, calculated based on modeled ozone concentrations in Northwestern Europe is shown in Figure 2 on a 0.5x0.5 degree grid level. PAF values varied between 0.00-0.30 for different species groups and effect definitions. The values indicate that in some regions potentially 13% of the perennial grassland species and 30% of annual grassland species have growth reductions of at least 10% when exposed to ambient ozone concentrations equivalent to those of 2010. From these maps it can be seen that continental Europe has the highest PAFs.

Figure 2

DISCUSSION

We derived SSDs for effects of ozone exposure on natural vegetation. Species were grouped according to endpoint (biomass decrease or no decrease) and taxonomy (trees, and annual and perennial grassland species). Both critical levels and spatially explicit impacts were determined. In the following, we discuss the main factors driving uncertainties regarding the AOT40-based effect data and extrapolation of data. After that, the results are interpreted and the application of SSDs in ozone risk assessment is discussed.

Uncertainties

Here, the concentration-based AOT40 method was used to estimate the risk of damage by ozone to natural vegetation. The use of the time integrated AOT40 index could lead to biases when the duration of exposure is very different from the model context where it is applied. In our study, however, the exposure duration and the modeled range of AOT40 are in line with each other. We used linear response models to describe species-specific ozone effect relationships. Such relationships are generally reported for crops in open top fumigation experiments (Musselman et al., 2006). However, for trees and semi-natural grassland communities non-linear response models have also been used to describe ozone exposure-effect relationships (Fuhrer et al., 1997; Manes et al., 2005). In particular, some studies have shown that perennial plants can have a non-linear response to long term ozone exposure of >2 yrs (Matyssek et al. 2003). These effects, however, are not yet fully understood because most fumigation experiments run for only 1 growing season (Kitao et al. 2009). Nevertheless, we have chosen to use linear exposure-response functions to determine our EC_{10} values because of the availability of data. The species-specific exposure-response relationships were directly taken from the literature and the number of data points in the published regressions differed widely between the species involved (3 to 145, 7 on average). A number of regressions have low R2 values for perennial and annual grassland species. As a sensitivity check, we derived HC_5 values only using species response curves with respectively R2 > 0.5 and R2 > 0.75 as cut off criteria (table S5). We found that the HC_5 values for the subselection of species with relatively high R2 values are not statistically different from the HC5 values based on all species information Moreover, some functions were based on a single experiment, hereby leading to an over- or underestimation of the response of individual plants to ozone. Furthermore, it is not known how representative exposure-response relationships determined in fumigation experiments using tree seedlings or saplings are for mature trees. There are conflicting reports in the literature as to whether saplings are more sensitive, less sensitive or of similar sensitivity to mature trees (e.g. Braun et al., 2007; Karnosky et al., 2007). In this study we use the tree response functions as a comparison to the grassland species and acknowledge that there are uncertainties in extrapolating to perennial mature trees.

In this study, only data from experiments using exposure systems close to natural conditions have been used, and results from closed chamber studies were excluded. A general concern is that the sensitivity to ozone exposure can be overestimated at the community level due to a bias towards the use of sensitive species in fumigation experiments (Mills et al., 2007b). Although OTC experiments are designed to expose species to ozone under natural conditions, differences in microclimate between the chamber-grown plants and those growing outside may lead to differences in plant response to the same exposure concentration (Pleijel et al., 1994). In addition, this study only considered above-ground biomass responses, whereas there could have been effects on below-ground biomass for some species (e.g. Wagg et al., 2012). Also, treatment of the plants, e.g. through watering, may alter plant sensitivity to pollutants (Fuhrer et al., 1997). Furthermore, environmental conditions and inter- and intraspecific variation in response to ozone exposure make the generic applicability of the SSDs difficult (Biswas et al, 2008; Staszak et al., 2004). Some climatic factors such as high vapour pressure deficits can reduce ozone uptake through stomata. (Grunhage et al., 1997). This can lead to an overestimation of the PAF and HC_5 values related to ozone. However, high temperature and VPD conditions are comparatively rare in northern Europe and in this region climatic conditions are favorable for ozone uptake (Mills et al., 2011) and we therefore consider the concentration-based approach used in this study to be valid in this region. The current SSDs are based on a Northwest European species composition; therefore it is not possible to give an accurate prediction of the ozone effects in other regions in Europe (Paludan-Muller et al., 1999). Because of these uncertainties the geographical domain of the application of our SSDs is limited to Northwestern Europe. Flux-based ozone exposure experiments can take into account environmental conditions which are closer to observed conditions compared to the AOT40-based exposure experiments used in the current analysis (Grunhage et al., 2003; Matyssek et al. 2007). If flux models for more species become available, the SSD-concept can also be applied with stomatal flux-based exposure-response data.

The SSD concept, however, has limitations (Forbes and Forbes, 1993; Forbes et al., 2001). The relative frequency of different life-cycle types, the proportions of sensitive and insensitive taxonomic groups in communities and the role of density-dependent influences on population dynamics are not considered in the SSD concept, but are potentially important to develop sound environmental quality criteria. Competitive and facilitative interactions among plants as well as among plants and soil organisms have the potential to modify both the direction and magnitude of the O3 response (Evans & Ashmore, 1992, Hayes et al., 2010). However, some studies have clearly demonstrated that the effects of ozone in species mixtures also can be greater than those on species grown alone or only subject to intraspecific competition (Grantz and Shrestha, 2006). A few studies have experimentally assessed the ecological significance of ozone exposure in grassland under field conditions. For example, Wedlich et al. (2012), indicate that ozone exposure in mesotrophic grassland significantly decreased the biomass of the herb fraction, however, no ozone effect was found for the grass component. They identified ozone as a dominant factor influencing species composition of the grassland community. Thwaites et al. (2006) demonstrated significant changes in species dynamics and composition in calcareous grasslands, both with positive and negative effects of ozone on different species, although total biomass and cover was not affected by ozone. Furthermore, some studies show that the species' O3 sensitivity is smaller and less frequent when plants are exposed in the field than expected from results derived from open top experiments (Bassin et al., 2007b; Stampfli & Fuhrer, 2010). On the other hand, these arguments apply as well to the SSD approach as to current critical levels, and are broad issues in all risk assessment approaches in the absence of almost any long-term community experiments in the field for grasslands.

Interpretation

The mean values of the SSDs were significantly lower for annual than for perennial grassland species. This indicates that annual grassland species, as a species assemblage, are more sensitive to ozone than perennial grassland species. This result can be explained by differences in life cycle, i.e. annual species are generally fast growing and therefore have higher stomatal flux and consequentially larger uptake of ozone (Bassin et al., 2007a; Hayes et al. 2007). Significant differences in variances were found for perennial grassland species and trees. These results can be explained by the relative small sample used to derive the SSD for trees, i.e. more species can give more variance in sensitivity. Furthermore, trees, as a species group, are more homogeneous with regard to the number of different plant families they represent (Musselman et al., 2006). However, it should also be considered that data was only available for comparatively few tree species.

The species selection, i.e. species with a biomass reduction only or all species, to determine critical ozone levels is guided by the protection objective. Conceptually, including all species in the SSD gives a more complete picture of ozone impacts on plant species communities. Statistically, however, no differences in critical levels were found between the different response types, indicating that the suggested conceptual differences between the response types have little influence on the critical ozone levels of a species group.

HC_5 values derived in this study are lower than the equivalent critical levels recommended by the LRTAP Convention (2010). Therefore, according to the standards of conventional ecotoxicology, plant species may not be sufficiently protected with current critical levels as > 5% of species within a community may be affected at concentrations less than the current critical levels. However, the choice for the protection level of 95% of the species remains somewhat arbitrary. This may explain why the levels derived in this study are lower than current critical levels for ozone.

This study indicates that up to 20% of the species will have a 10% biomass reduction due to ambient ozone exposure. Unfortunately not enough long-term field observational studies on community level impacts of ozone exposure are available to verify the PAFs corresponding to modeled ozone concentrations (Bassin et al., 2007a; Klingberg et al., 2011). Our results of ozone impact do not fully reflect actual changes in species composition, because changes in competition and species dynamics are not taken into account. The PAF specifies the potentially affected fraction of species by ozone exposure and not the actually affected fraction.

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SUPPLEMENTARY INFORMATION

Table S1: Exposure response functions perennial grassland species

Table S2: Exposure response functions annual grassland species

Table S3: Exposure response functions trees species

Table S4: Statistical testing species classes

Table S5: HC_5 values at different R2 cutoffs

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Table 1. Means (μ) and standards deviations (σ) of HC_5 for trees, annual grassland species and perennial grassland species, based on EC_{10}-data for the individual species within the group, HC_5 values in ppm.h (90% confidence interval) and PAF values corresponding to the critical level (90% confidence interval).

		n species	μ	σ	HC_5	Critical level[1]	PAF calculated current critica levels of ozon
Annual grassland species	Biomass reduction only	22	0.84	0.42	1.37 (0.75-2.09)	3	0.20 (0.10-0.28)
	Fraction no biomass decrease	25	0.84	0.42	1.67 (0.81-2.58)	3	0.17 (0.09-0.30)
Perennial grassland species	Biomass reduction only	39	1.14	0.47	2.33 (1.59-3.19)	5	0.17 (0.09-0.30)
	Fraction no biomass decrease	62	1.14	0.47	2.81 (1.77-4.13)	5	0.11(0.06-0.21)
Trees	Biomass reduction only	9	1.10	0.29	4.10 (1.72-6.58)	5	0.08 (0.01-0.28)

[1]Critical levels based on the AOT40-based method determined by LRTAP convention 2010.

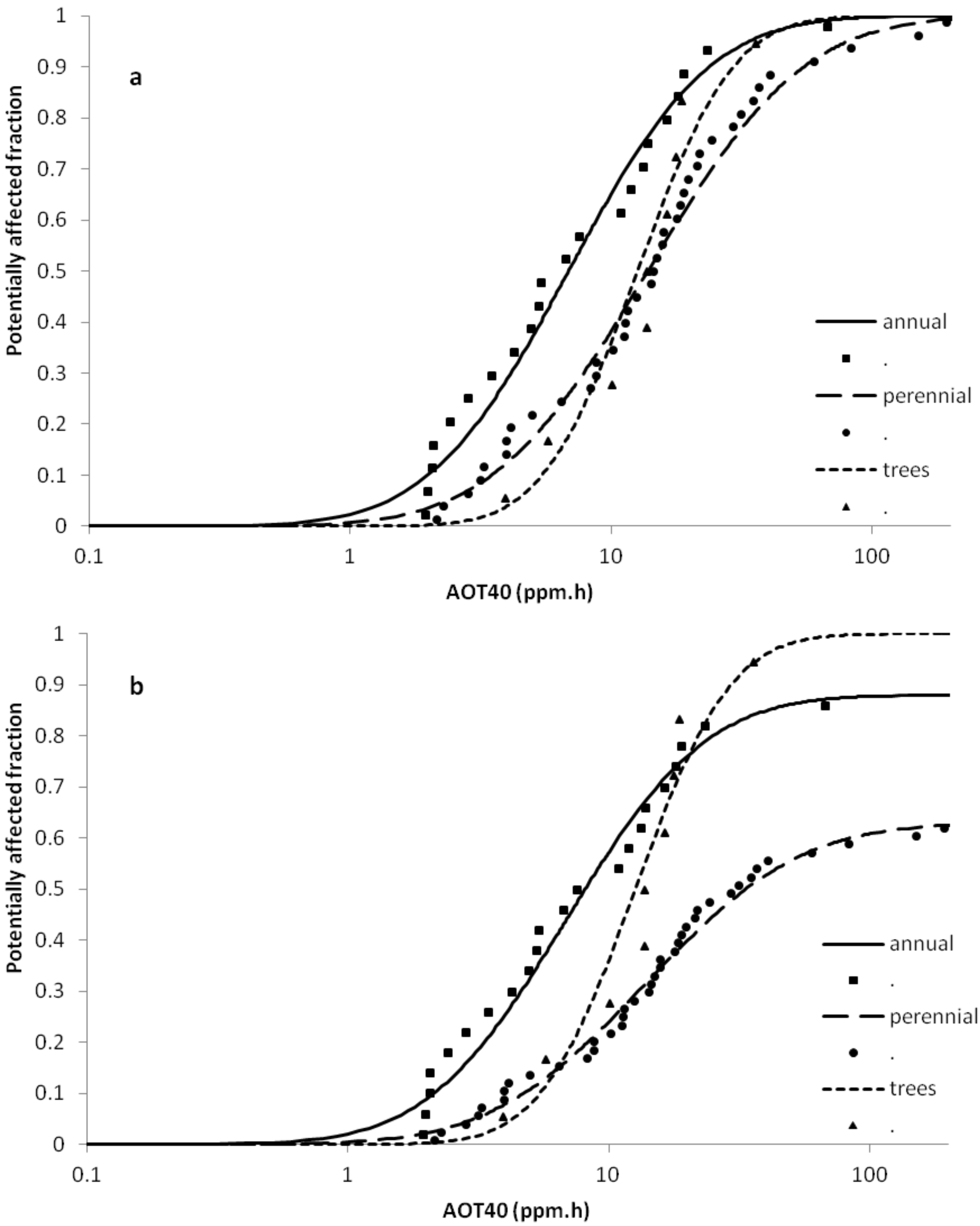

Figure 1. Species sensitivity distributions for annual grassland species (solid line), perennial grassland species (dotted line) and trees (finely dotted line) based on biomass reduction only (a) and with the fraction of species with no biomass decrease included (b).

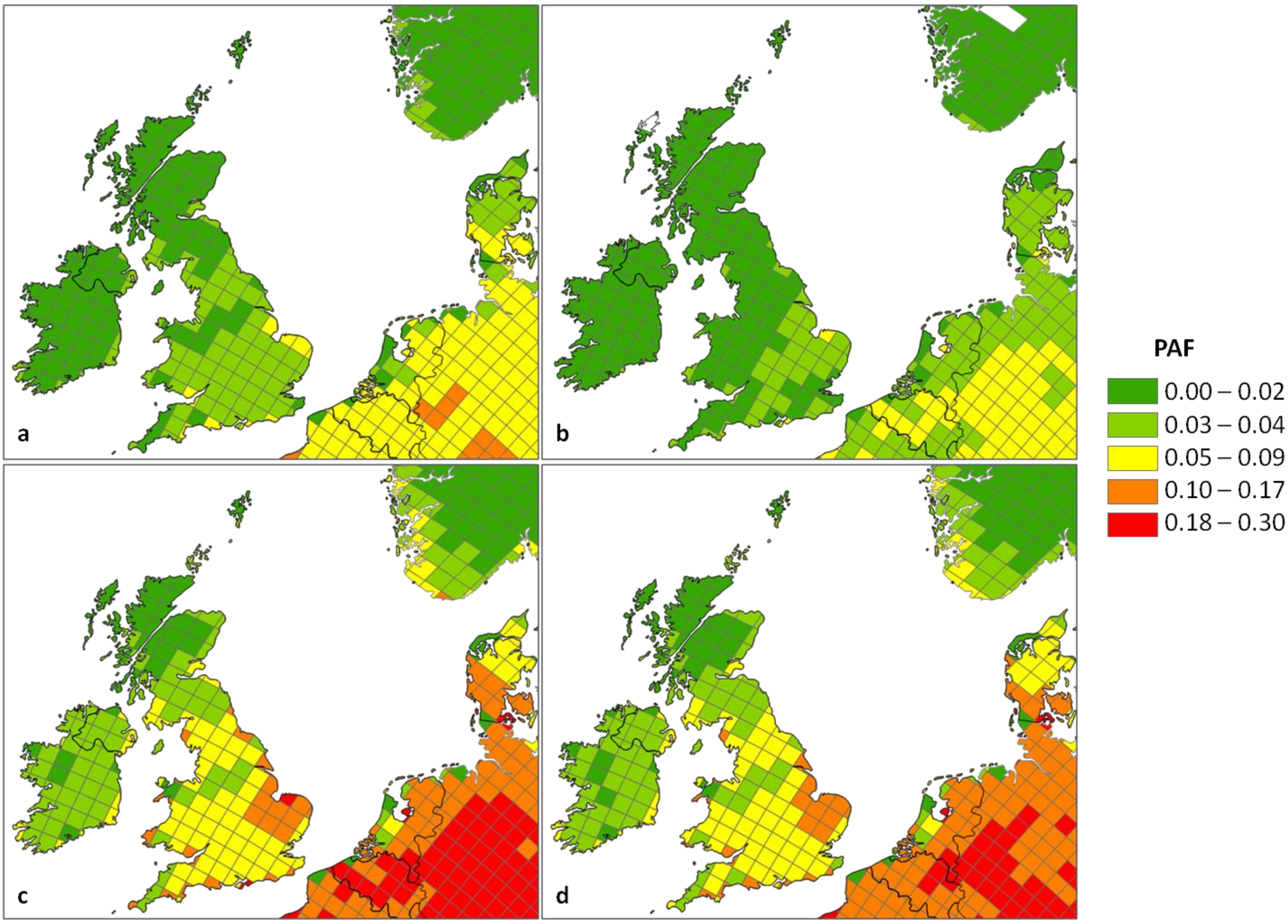

Figure 2. The potential affected fraction corresponding to modeled ozone levels (AOT40 in 2010) for perennial grassland species using biomass reduction only (a) and including the fraction of species with no biomass decrease (b), and for annual grassland species using biomass reduction only (c) and including the fraction of species with no biomass decrease (d).