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Ozone critical levels for (semi-)natural vegetation dominated by perennial grassland species

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Abstract

New critical levels for ozone based on accumulated flux through stomata (phytotoxic ozone dose, POD), for temperate perennial grassland (semi-)natural vegetation have been agreed for use within the Convention on Long-Range Transboundary Air Pollution. These were based on data from several experiments conducted under naturally fluctuating environmental conditions that were combined and analysed to give linear dose-response relationships. Dose-response functions and flux-based critical levels were derived based on biomass and flower number. These parameters showed a statistically significant decline with increasing accumulated stomatal ozone flux. The functions and critical levels derived are based on sensitive species and can be used for risk assessments of the damaging effect of ozone on temperate vegetation communities dominated by perennial grassland species. The critical level based on flower number was lower than that for biomass, representing the greater sensitivity of flower number to ozone pollution.

Keywords

Ozone; biodiversity; flowering; stomata; critical level; biomass

Introduction

Tropospheric ozone is a secondary pollutant formed by reactions of precursors, which mainly originate from anthropogenic sources, in the presence of sunlight. Baseline ozone concentrations increased rapidly during the late 20th century and at northern mid-latitudes approximately doubled over the period 1950 to 2000 (Parrish et al., 2012). These levels caused significant damage to vegetation such that impacts could be observed on plants in ambient air conditions, with field evidence of effects demonstrated on over 80 (semi-)natural vegetation species in 16 European countries over the period 1994-2006 (Mills et al., 2011a). Since approximately 2000, the rate of increase in ozone concentration has slowed, and for individual monitoring sites in Europe there can be decreasing, increasing or no trend in summertime daytime ozone concentrations over the period 2000–2014 depending on the individual site (Chang et al., 2017). This is thought to reflect changes in emissions of ozone precursors such as carbon monoxide, methane, non-methane volatile organic compounds and nitrogen oxides. Many models of future ozone projections are very sensitive to small changes in emissions and/or climate change (Young et al., 2013). Despite some efforts to reduce emissions of precursor molecules in many parts of Europe, model results from the Task Force on Hemispheric Transport of Air Pollution show that for ozone in Europe, the contributions from precursor emissions originating from the rest of the world are larger than for European emissions alone, with the largest contributions from North America and eastern Asia (Jonson et al., 2018).

Ozone enters plants through stomata. Two of the factors that influence sensitivity to ozone are control of the flux of ozone into the leaf, and the capacity for detoxification and repair processes (Wieser and Matyssek, 2007). Ozone damage can trigger a cascade of defence reactions, which can further affect plant responses including growth or resource partitioning (Weigel et al., 2015). Many species of (semi-)natural vegetation are known to be sensitive to ozone pollution, based on ozone-exposure experiments and can show significant impacts with average ozone concentrations less than 70 pbb (e.g. Hayes et al., 2012, Rämő et al., 2007). Effects can include visible leaf injury, reduced flowering, reduced seed quality, premature senescence and reduced plant or seed biomass, however, some species have been shown to be resistant to ozone (Bungener et al., 1999, Hayes et al., 2006).

Semi-natural vegetation types including grassland, heathland and fens have been identified as sensitive to ozone pollution based on the responses of the component species (Mills et al., 2007). A recent review by Bergmann et al. (2017) showed that 258 herbaceous species assessed during field studies along ozone gradients in Europe and North America showed visible injury symptoms attributed to ozone pollution. Evidence from simple constructed species mixtures has shown changes to the plant community including alterations in the biomass of individual component species (Rämő et al., 2007), reductions in total biomass (Hayes et al., 2010) and alterations in flower number and timing of flowering (Hayes et al., 2012). Results from intact plant communities is scarce, but effects have been shown. These include effects on species composition without an accompanying change in total biomass of an upland mesotrophic grassland (Wedlich et al., 2012) and a calcareous grassland (Thwaites et al., 2006). However, some other studies have shown small or no effects (Rinnan et al., 2013; Volk et al., 2014), sometimes despite containing some individual species that are known to be sensitive to ozone.

Analysis of survey data in the UK has shown changes in grassland species composition that were partially attributed to ambient ozone (Payne et al., 2011). Impacts of ambient ozone have also been reported in the field in Europe (Mills et al., 2011a), USA (Fuhrer et al., 2016; Temple et al., 2005; U.S. Environmental Protection Agency, 2014) and south-east Asia (Emberson et al., 2009, Feng et al., 2015), based on occurrence of visible leaf-injury symptoms and air-filtration and chemical protection experiments investigating injury and/or yield. Beneficial effects of reducing ambient ozone concentrations using filtered air have been shown for some vegetation types, particularly for crops such as wheat (Pleijel et al., 2018) and spinach (Gonzalez-Fernandez et al., 2016), and also for (semi-)natural vegetation species (Gimeno et al., 2004; Sanz et al., 2011). The meta-analysis of Pleijel et al. (2018) showed that a range of yield and growth variables responded negatively in non-filtered compared to charcoal-filtered air, with the mean ozone concentration of the non-filtered air treatments being 36 ppb.

Critical levels for ozone for (semi-)natural vegetation based on reductions in biomass were first proposed in a workshop in Kuopio in 1996 (Kärenlampi and Skärby, 1996) and were based on a limited range of experimental data collated by Ashmore and Davison (1996). There has been an increasing body of evidence to demonstrate that fluxes of ozone into the leaves of vegetation is a better metric to predict risk of damage to vegetation than ozone concentration (e.g. Mills et al., 2011a). In order to calculate fluxes of ozone to vegetation it is necessary to parameterise a model to describe the extent of opening of stomata in response to changing meteorological conditions (Emberson et al, 2000), which requires a dataset of measurements of stomatal conductance. Flux-based critical levels were approved and documented by the Convention on Long-Range Transboundary Air Pollution (LRTAP Convention, 2010), providing a standardised methodology for application at national and regional scales. A total of eight flux-based critical levels were agreed in 2010 (Mills et al., 2011b). For (semi-)natural vegetation, a single critical level was based on the response of *Trifolium spp.*, for protection of pasture and fodder quality in productive perennial grasslands, and the vitality of natural species in perennial grasslands of high conservation value (Mills et al., 2011b).

Since 2010, data have become available for a wider range of (semi-)natural vegetation species, and encompassing a wider range of response variables. In particular a growing number of experimental work has included accompanying stomatal conductance measurements, allowing parameterisation of stomatal flux models. Due to the large diversity of (semi-)natural communities across Europe in terms of ecophysiology, life form, species composition and management practices such as grazing, cutting or fertilisation regime, ozone critical levels have been established for widespread ozone sensitive species representing broad categories of (semi-)natural vegetation plant communities. Here we provide an overview of three new flux-based critical levels of ozone for (semi-)natural vegetation and the response functions used to derive them. These functions are suitable for use in temperate perennial grasslands found in Boreal, Atlantic and Continental biogeographical regions of Europe that are dominated by grasses and forbs and have little or no tree cover, and may be grazed. These were agreed for use within the Convention on Long-Range Transboundary Air Pollution (CLRTAP) at the 30th Task Force Meeting of the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) in 2017. Details of flux-based critical levels of ozone for other vegetation, including Mediterranean annual grassland, temperate and Mediterranean crops and trees, and methodology for applying these critical levels in risk assessments is found in LRTAP Convention (2017).

Methods

Data used

Data were collated from northern/mid European pasture and grassland experiments, from free-air ozone enrichment, open-top chamber and solardomes experiments conducted with intact swards, constructed mixed species communities or individual species, exposed to ozone for between 2 and 6 months. For each experiment, data on the response of individual species was used rather than the total biomass of a mixed species community in order to avoid missing effects where changes in species composition had occurred, but with no change in total biomass (Thwaites et al., 2006; Wedlich et al., 2012). The complete database comprised a total of 39 experiments, of which 30 were published and 9 used data from unpublished studies (Supplementary material).

Calculation of POD_1SPEC

POD_1SPEC is a species-specific phytotoxic ozone dose accumulated over a threshold of y. Where necessary hourly data and species-specific parameterisations were available, stomatal flux for ozone was modelled for each species in each experiment using DO_3SE version 3.1. The model is described in Emberson et al. (2000) and uses a multiplicative algorithm based on reduced stomatal opening in response to light, temperature, soil moisture, vapour pressure deficit (VPD), ozone concentration and plant phenology (e.g. anthesis, seed formation), compared to the stomatal conductance under optimum conditions. Stomatal ozone fluxes were calculated based on projected leaf area (PLA). No attempt was made to estimate the soil moisture in some experiments/treatments and thus only well-watered treatments were included to be able to assume that soil moisture did not affect the stomatal uptake of ozone.

Initially stomatal ozone fluxes were calculated based on species-specific models, however, due to the high variability in g_{max} between species, it became apparent when combining data from different species, that any comparison was overwhelmed by the influence of g_{max} on total stomatal ozone flux. In addition, the environmental response of stomatal ozone flux between the grassland species was broadly similar (Supplementary material). Hence, 'standardised' total stomatal ozone flux was calculated, using a fixed g_{max} of 190 mmol O_3 m^{-2} PLA s^{-1} for grasses and 210 mmol O_3 m^{-2} PLA s^{-1} for forbs/legumes, representing the mean values from the individual species parameterisations (supplementary material). In addition, a common parameterisation was used for response to meteorological variables, separately for 'grasses' and 'forbs/legumes' (Table 1). This approach also allowed a wider number of species to be included in the analysis, as species-specific stomatal ozone flux models and g_{max} values were not available for all species.

It is generally accepted that plants can detoxify a certain amount of ozone, and it is the ozone above this threshold that is harmful, therefore, a threshold should be used in calculations of phytotoxic ozone dose (Musselman et al., 2006). In order to account for an ozone detoxification threshold of the plants when calculating the POD, a flux-threshold of 1 nmol m^{-2} s^{-1} was used for both 'grasses' and 'forbs/legumes' ('$_y$' in POD_y). The threshold of 1 nmol was tested for suitability according to the recommendation made by Büker et al. (2015), i.e. that for the normalised linear response function the confidence interval of the intercept includes 100%. The value of 1 nmol is further supported as this matches the flux-threshold used to establish flux-based ozone critical levels for forest trees.

POD_1SPEC was calculated for all species in each experiment where hourly ozone and meteorological data were available, using either the 'grasses' or 'forbs/legumes' parameterisation as appropriate. Relative biomass based on POD_1SPEC for each species in each experiment was calculated, and datapoints with relative biomass>2 were omitted as outliers. Those with relative biomass <0.4 were checked for plausibility by assessing whether they were outlier points, or were a component of the response relationship of a sensitive species.

<u>Ozone critical levels</u>

Critical levels for ozone are designed to protect the most sensitive species (LRTAP Convention, 2010), therefore, data from individual species were included in subsequent analysis subject to meeting the following criteria: a) number of datapoints >3, b) intercept of POD_1SPEC relationship between 0.9 and 1.1, and c) slope of relationship < median of all negative slopes. Points 'a' and 'b' are applied together to ensure that there is a usable relationship between POD_1SPEC and the response parameter. Point 'c' is to ensure that analysis is based on sensitive species.

When using low flux thresholds 'y' of 1 nmol m^{-2} s^{-1}, ozone concentrations as low as 3-5 ppb can contribute to ozone flux (González-Fernández et al., 2016; Hayes et al., 2019a), far below background levels and even far below estimated ozone concentrations in the mid-19th century. Therefore, it is not appropriate to use a POD_1 of zero for deriving ozone critical levels. Hence, at the CLRTAP Ozone Critical Level Workshop in Madrid in November 2016, it was decided that an accumulated flux value calculated at a constant 10 ppb ozone (estimated pre-industrial mean ozone concentration) should be set as a reference value (Ref10 POD_Y) for determining flux-based critical levels, by calculating POD_Y using a constant ozone concentration of 10 ppb and the climatic conditions in the experiment. If data from several experiments were combined from different climates, the mean of the Ref10 POD_Y was used as the Ref10 POD_Y for that function (Scientific Background Document A of LRTAP Convention, 2017). The reference

PODy represents a minimum ozone flux that would be achieved by a plant species or community under clean air conditions. To calculate critical levels of stomatal ozone flux for an effect using POD_1SPEC, a 10% reduction in the effect parameter was used, compared to that at $Ref10POD_1$. The application of this approach in the derivation of a critical level is shown in Figure 1. The use of Ref10 PODy does not affect the slope of the dose-response relationship, it is used purely for the purposes of setting Critical Levels of ozone, because otherwise there could theoretically be an ozone critical level that is not achievable, with the ozone concentrations needed for this lower than pre-industrial ozone. Even in pre-industrial times there were ozone concentrations estimated to be 10-15 ppb (Royal Society, 2008).

Statistical Analysis

Analysis of the relationships between POD_1SPEC and the response parameters of 'above-ground biomass', 'total biomass' and 'flower number' was performed using linear regression in R (R Core Team, 2018). Graphical model validation was carried out using plots of the standardised residuals, to ensure that there was no evidence of heterogeneity.

Table 1: Parameterisation table for the DO_3SE model for POD_1SPEC calculations for sunlit leaves at the top of the canopy for representative (semi-)natural vegetation species of temperate perennial grasslands. Full definitions of the parameters are provided in LRTAP Convention (2017).

Parameter	Units	(Semi-)natural vegetation parameterisation for sunlit leaves at top of canopy - POD_1SPEC	
Region *(may also be applicable in these regions)*		Atlantic, Boreal, Continental, *(Pannonian, Steppic)*	
Land cover type		Perennial grasslands (Grass spp.)	Perennial grasslands (Forbs incl. legumes)
g_{max}	mmol O_3 m^{-2} PLA s^{-1}	190	210
f_{min}	fraction	0.1	0.1
light_a	-	0.01	0.02
T_{min}	°C	10	10
T_{opt}	°C	24	22
T_{max}	°C	36	36
VPD_{max}	kPa	1.75	1.75
VPD_{min}	kPa	4.5	4.5
ΣVPD_{crit}	kPa	-	-
PAW_t	%	-	-
SWC_{max}	% volume	-	-
SWC_{min}	% volume	-	-
SWP_{max}	MPa	-0.1	-0.1
SWP_{min}	MPa	-1	-0.6
f_{O3}	fraction	-	-
A_{start}_FD[i]	day of year	91 (April 1st)	91 (April 1st)
A_{end}_FD[i]	day of year	273 (September 30th)	273 (September 30th)
Time window length	month	3	3
Leaf dimension	cm	2[ii]	4[ii]
Canopy height	m	0.2	0.2
f_{phen_a}	fraction	1	1
f_{phen_b}	fraction	1	1
f_{phen_c}	fraction	1	1
f_{phen_d}	fraction	1	1
f_{phen_e}	fraction	1	1
$f_{phen\_1\_FD}$	no. of days	-	-
$f_{phen\_2\_FD}$	no. of days	-	-
$f_{phen\_3\_FD}$	no. of days	-	-
$f_{phen\_4\_FD}$	no. of days	-	-
LIM_{start_FD}	year day	-	-
LIM_{send_FD}	year day	-	-

"-" = parameterisation not required for this species.

[i] *Days of year given for non-leap year. Note that this refers to the start and end of the accumulation period based on a fixed day rather than effective temperature sum.*

[ii] *Not given, set to match wheat (grass species) and potato (forb species, including legumes).*

Primary abbreviations for the parameters are temperature (T), vapour pressure deficit (VPD), plant available water (PAW), soil water content (SWC), soil water potential (SWP). Fphen is used to define the phenology function.

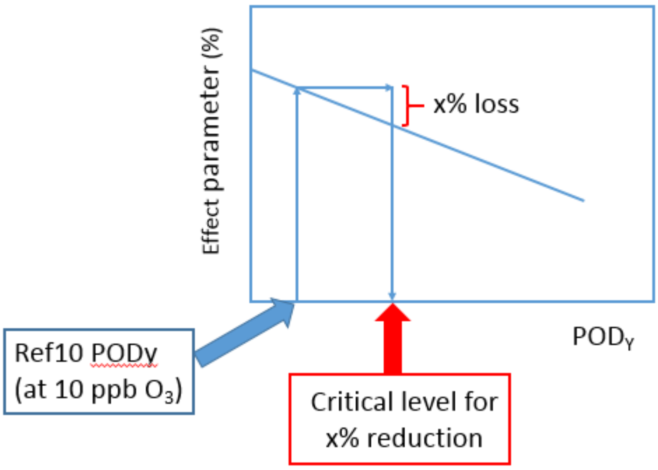

Figure 1: Method for using Ref10 POD_Y (i.e. POD_Y at 10 ppb constant ozone) as reference point for ozone critical level derivation.

Results

Dose-response functions for above-ground biomass and total biomass, based on POD_1SPEC and using a standardised g_{max}, are shown in Figure 2a and 2b. Only ozone-sensitive species are included and the number of datapoints varied between the two figures, as not all component experiments measured root biomass in addition to shoot biomass. The number of species used to derive these functions was six and four for above-ground biomass and total biomass respectively. The relationships are statistically significant, with $p=0.018$ for above-ground biomass and $p<0.001$ for total biomass. Comparison of the slope of the response function shows that the relationship for above-ground biomass is not statistically more sensitive to ozone than that for total biomass ($p>0.05$). Relative flower number shows a significant decline with increasing ozone dose ($p<0.001$; Figure 2c). The relationship for flower number is based on 32 datapoints from four ozone-sensitive species. The total list of species in the final response functions comprises: *Campanula rotundifolia*, *Dactylis glomerata*, *Fritillaria meleagris*, *Leontodon hispidus*, *Potentilla erecta*, *Primula veris*, *Ranunculus acris*, *Sanguisorba officinalis*, *Sanguisorba minor*, *Scabiosa columbaria*, *Trifolium pratense*. Figures illustrating which species is represented by each datapoint are shown in the Supplementary material.

For each of these relationships the corresponding reference flux, REF10 POD_1, was calculated as 0.1 mmol m^{-2} PLA. A 10% reduction in the effects parameter was considered to be a biologically significant effect that could be important for plant ecoystems. Therefore, critical levels were determined for a 10% reduction of the effect based on the slope of the relationship. The critical level is lowest for flower number (6.6 mmol m^{-2} PLA) and highest for total biomass (16.2 mmol m^{-2} PLA), with the critical level for above-ground biomass intermediate (10.2 mmol m^{-2} PLA).

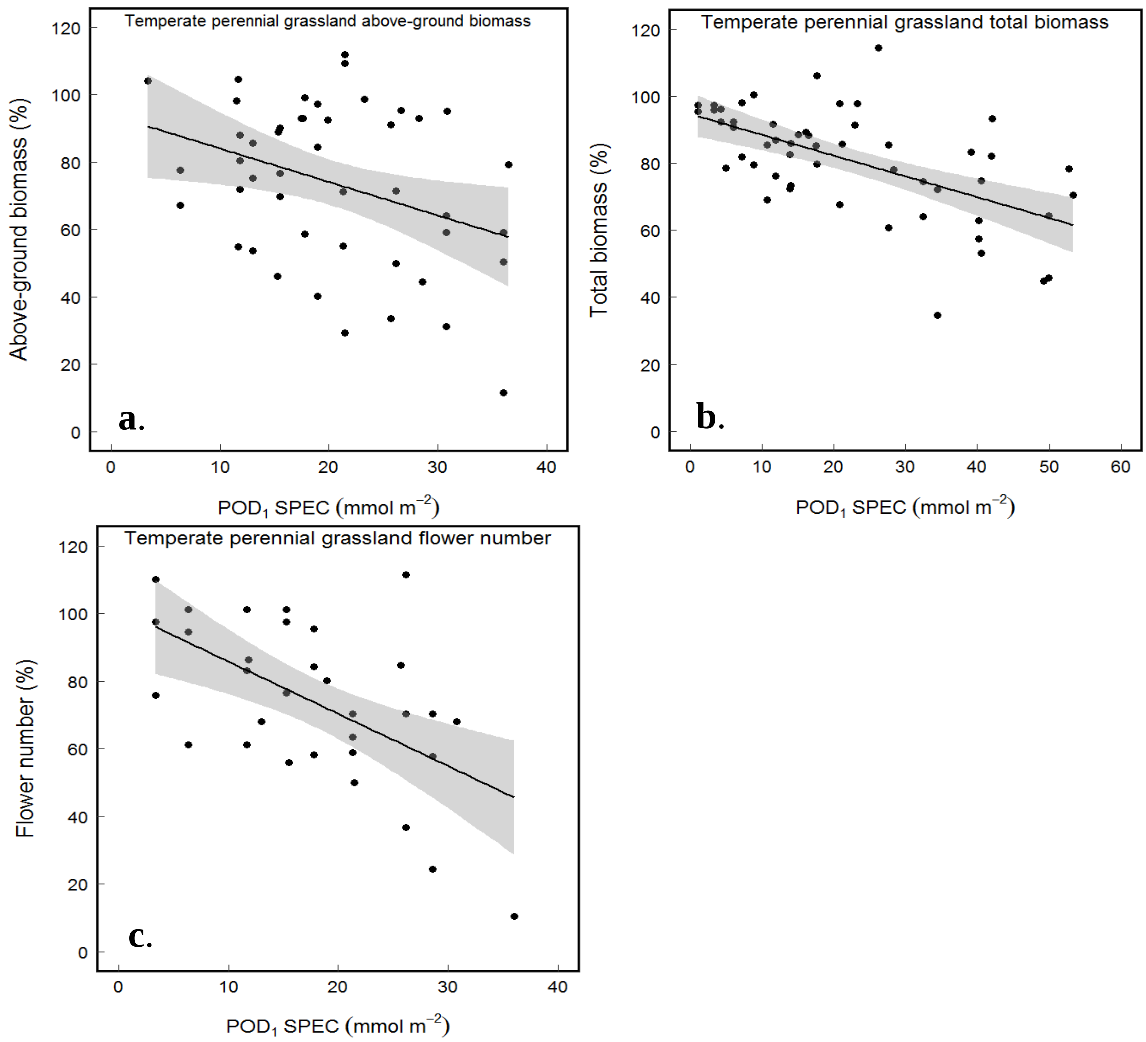

Figure 2: Ozone flux-effect relationships based on POD_1SPEC for temperate perennial grasslands for a) above-ground biomass, b) total biomass and c) flower number. The statistical significance of the slopes are $p=0.018$, $p<0.001$ and $p<0.001$ respectively.

Discussion

Species tested and representativeness of perennial grassland ecosystems in Europe

There are 4000+ species of (semi-)natural vegetation in Europe. Although response functions and relative sensitivities have been derived for >100 species (Hayes et al., 2007; Bergmann et al., 2015), at least 98% of (semi-)natural species remain untested. A larger range of species, and habitat coverage is needed in order to improve the assessment of risk from ozone pollution to (semi-)natural vegetation. The use of biogeographic regions (EEA, 2016) is a tool that can be used to define the area for which a critical level is applicable means that the critical levels can be used over a wider area than from where the experimental data originated (LRTAP Convention, 2017). This is useful for identifying areas that might be at risk of adverse effects of ozone pollution, but assumes that the same species are found across the biogeographic region.

The most suitable EUNIS classes (http://eunis.eea.europa.eu/) to use are those represented by the habitats for which the critical levels have been derived. These include the temperate perennial grasslands ‘Permanent mesotrophic pastures and aftermath-grazed meadows’, ‘Low and medium altitude hay meadows', 'Unmanaged mesic grassland' and 'Meadows of the steppe zone'. However, not all temperate grasslands are sensitive to ozone, for example, alpine grasslands and pastures are known to be more resilient to ozone in terms of biomass growth (Volk et al., 2014). These critical levels are applicable to perennial grasslands in the European biogeographic regions 'Boreal', 'Atlantic' and 'Continental', as the functions were derived from species found in these regions (LRTAP Convention, 2017). They may also be indicative of risk in Steppic and Pannonian grasslands as these species can also occur in these regions, although they may be of smaller importance.

Comparison to parameterisations and CL's from the Mediterranean region

The critical levels derived here for temperate perennial grasslands were of a similar magnitude to those proposed for annual Mediterranean pastures, where the critical levels proposed for a 10% loss of above-ground biomass and reproductive capacity were a POD_1SPEC of 12.2 mmol m^{-2} and 7.2 mmol m^{-2} respectively (Sanz et al., 2016). As for temperate perennial grasslands, reproductive capacity was more sensitive to ozone than biomass.

Indicators of biodiversity and ecosystem services

In this study flower number was used as a proxy for biodiversity, and biodiversity was not measured directly. Other relevant indicators could include seed quality as a reduction in seed germination rate by up to 30% has been shown for ozone-treated plants compared to control with ozone concentrations of 55 ppb (8h daily mean, Bender et al., 2006). Relative abundance of individual vegetation species within a community has also been shown to be affected by ozone (Evans and Ashmore, 1992; Wedlich et al., 2012), and the response of a dominant species in the community could also influence biodiversity, as secondary effects on species composition have been shown in some studies (Thwaites et al., 2006).

In the current study the critical level for flower number was lower than that for biomass, indicating the high sensitivity of this biologically significant response variable. Although there are comparatively few studies on (semi-)natural vegetation species, similar patterns have been demonstrated in some other vegetation,, for example, effects of ozone on wheat grain yield were larger than the effects on biomass, based on a combined analysis of 22 experiments (Pleijel et al., 2014). Similarly, a study based on 128 experiments across all vegetation types (including crops) showed that elevated ozone corresponded to a reduction in fruit number, fruit weight and seed number, and concluded that detrimental effects of ozone on reproductive growth and development were compromising the fitness of native plant species (Leisner and Ainsworth, 2012). Leisner and Ainsworth (2012) also showed that there was a decrease in the flower number with elevated ozone and that this was larger for perennial plants than for annuals. This could be due to a change in biomass partitioning, as there could be enhanced allocation to repair and maintain leaves damaged by ozone, particularly in perennial species, in order to maintain photosynthetic capacity to sustain longer-term survival.

Direct effects on animals, fungi, bacteria and insects that live in close association with these plants have not been considered as there is only a small amount of quantified evidence for these impacts (Bergmann et al., 2017). The dose-response relationships described in this study could also be used as a starting point for assessing the risk from ozone pollution on ecosystem services, for example, effects on total biomass and above-ground biomass could indicate potential effects on carbon sequestration.

Interactions with other factors

In deriving response functions, the impact of ozone was considered in isolation. However, vegetation is exposed to multiple stresses that could have ameliorating or exacerbating interactions and theses interactions are complex so that responses cannot be extrapolated from responses to single drivers. Climatic conditions, rising carbon dioxide and other pollutants can modify the responses of vegetation to ozone. Some of these influence the amount of ozone uptake through the stomata and therefore can be partially accounted for by using POD as the ozone exposure metric, for example changes in meteorological inputs such as warming (Bender and Weigel, 2007; Hayes et al., 2019a). However, changes in nitrogen input, including from atmospheric deposition, and changes in climate can also affect plant growth and biomass partitioning and these can act in combination with ozone. For example, at low ozone concentrations nitrogen can stimulate biomass, whereas this stimulating effect can be lost at high ozone concentrations (Wyness et al., 2011; Mills et al., 2016; Dai et al., 2019). In addition, nitrogen deposition can alter the species number and composition within plant communities, which may also alter community sensitivity to ozone pollution (Hayes et al., 2019b). Elevated carbon dioxide might reduce stomatal opening, but the response of vegetation to combined elevated carbon dioxide and ozone was found to be finely balanced depending on their relative concentrations in the atmosphere (Uddling et al., 2010). The timing of the growing season and therefore the timing of ozone uptake by the vegetation may also be affected by changes in climate (Peñuelas et al., 2002; Menzel et al., 2006).

Long-term effects

These relationships are based on relatively short-term responses of vegetation to ozone. Ozone pollution and regions of highest uptake can vary in spatial distribution between different years, depending on factors including local meteorology so that some areas may experience conditions favouring high ozone uptake intermittently rather than continuously (Hewitt et al., 2016). However, legacy effects can also occur, for example, spring biomass of plants exposed to ozone the previous year was reduced for some species (Hayes et al., 2006) and for soil with a history of elevated ozone, plant biomass was reduced compared to cores from ambient conditions (Li et al., 2015).

Use of the y threshold (and g_{max})

Use of a threshold ozone flux represents the ability to detoxify low amounts of ozone that enter the plant, with increased damage occurring when this is exceeded (Burkey et al., 2006). The mechanisms for this include antioxidants such as ascorbate and glutathione (Foyer and Shigeoka, 2011), which are produced by the plant and react with ozone to form less harmful products. It has been suggested that differences in sensitivity to ozone may be due to differences in either antioxidant concentration to scavenge free radicals (Zhang et al., 2012) or the ability to rapidly upregulate antioxidants and enzyme activity in the presence of stress (Wang et al., 2010). Using a constant threshold for stomatal ozone flux ('Y' in POD_Y: Phytotoxic Ozone Dose over a threshold flux of Y nmol m^{-2} PLA s^{-1}) is considered to act as a surrogate for an ozone detoxification threshold (Musselman et al., 2006) with different values used for different types of vegetation, i.e. crops, trees and (semi-)natural vegetation (Mills et al., 2011b).

Canopy-scale fluxes

Whilst this analysis assumes that all species in a grassland experience the same ozone uptake, the relative position of a plant within the vegetation canopy could affect both ozone concentration and the rate of ozone uptake. It has been shown that the vertical distribution of ozone within a grassland canopy is influenced by the vertical distribution of leaf area and turbulence, with ozone concentrations at 25 cm height reduced by 36% compared to the concentration at 90 cm (Jäggi et al., 2006). There could also be an influence of microclimate within the canopy, particularly for low-growing plants, and this can influence the ozone uptake due to effects of some variables (including light, temperature and humidity) on stomatal opening.

Within the DO_3SE model the ozone flux to leaves of the upper canopy is calculated, whereas the modelled flux to the whole canopy may be different, and also affected by factors including the frequency of hay cuts and timing of the growing season (Ashmore et al., 2007). In order to use canopy scale fluxes for ozone risk assessment, effects at the canopy scale need to be available from experiments. This should include changes in species composition, as there could be underestimations in assessment of risk if there is a change in species composition but not in overall biomass. Any change in species composition is also required to assess potential implications of ozone on biodiversity.

Conclusions

This paper provides an overview of the recently revised flux-based dose-response relationships and critical levels that have been agreed within the LRTAP Convention for use in assessment of risk to (semi-)natural vegetation dominated by temperate perennial grassland. For a 10% reduction in above-ground biomass, total biomass and flower number, the critical levels are a POD_1 of 10.2, 16.2 and 6.6 mmol m^{-2} respectively. Further work is needed to include a wider range of species and to cover a wider range of plant habitats. In addition, further work is needed to characterise the potential modifying influence of climate change and other pollutants on these response functions.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conceptualisation and design. Data collection and analysis was performed by FH, with guidance from JB, LG, HH and GM. The first draft of the manuscript was written by FH. All authors read and approved the final manuscript.

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Ozone critical levels for (semi-)natural vegetation dominated by perennial grassland species

Supplementary Information

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Table S1: References for the 39 studies included in the database of responses of (semi-)natural vegetation to ozone. Note that it was not possible to calculate ozone fluxes for many of these datasets due to lack of availability of hourly meteorological data. All studies except Kohut et al. (1988) and Pfleeger et al. (2010) used pots/large containers. All studies except the USA studies of Kohut et al. (1988) and Pfleeger et al. (2010) were conducted in Europe.

Reference	Experimental system	Approximate duration	Number of species
Bergmann et al, 1999. New Phytologist 144:423-435	OTCs	6 weeks	17
Bungener et al, 1999. New Phytologist 142:283-293	OTCs	23 weeks	24
Calatayud et al, 2011. Ecotoxicology and Environmental Safety 74:1131-1138.	OTCs	16 weeks	1
Hewitt et al, 2014. Environmental Pollution 189:111-117.	Solardomes	12 weeks	2
Danielsson et al, 1999. Environmental and Experimental Botany 42:41-49.	OTCs	7 weeks	2
Dawnay and Mills 2009. Environmental Pollution 157:503-510.	Solardomes	12 weeks	1
Foot et al, 1996. New Phytologist 133:503-511.	OTCs	26 weeks	1
Franzaring et al, 2000. Environmental and Experimental Botany 44:39-48	OTCs	14 weeks	10
Gimeno et al 2004 Environmental Pollution 132: 297-306	OTCs	10 weeks	19
Hayes et al. unpublished UK Harebell ploidy study 2012.	Solardomes	12 weeks	1
Hayes et al., 2006. Atmospheric Environment 40:4088-4097.	Solardomes	10 weeks	27
Hayes, Williams, Macmillan, Mills Unpublished UK data 2006.	Solardomes	12 weeks	6
Williamson et al., 2010. Environmental Pollution 158:1197-1206.	Solardomes	4 weeks	7
Jones et al 2010. Environmental Pollution 158:559-565.	Solardomes	10 weeks	1
Kohut et al 1988. Journal of Environmental Quality 17:580-585.	OTCs	12 weeks	2
Ramo et al., 2006. Environmental and Experimental Botany 58:287-298.	OTCs	12 weeks	1
Mortensen and Nilsen 1992. Norwegian Journal of Agricultural Science 6:195-204.	Growth Chambers	5 weeks	16
Pfleeger et al 2010. Agriculture, Ecosystems and Environment 138:116-126	OTCs	13 weeks	5
Pleijel and Danielsson 1997. New Phytologist 135:361-367.	OTCs	4 weeks	27
Power and Ashmore 2002. New Phytologist 156:399-408.	OTCs	3 weeks	12
Ramo et al 2007. Environmental Pollution 145:850-860.	OTCs	37 weeks	7
Sanz et al 2007. Atmospheric Environment 41:8952-8962.	OTCs	4 weeks	1
Sanz et al 2011. Environmental Pollution 159:423-430.	OTCs	8 weeks	1
Sanz et al 2014. Atmospheric Environment 94:765-772.	OTCs	5 weeks	1
Hayes et al, 2010. Atmospheric Environment 44:4155-4165.	Solardomes	24 weeks	7
Hayes et al, Unpublished UK data 2005.	Solardomes	12 weeks	8
Hayes et al, Unpublished UK data 2006 (individual species)	Solardomes	12 weeks	6
Hayes et al, Unpublished UK data 2006. (communities)	Solardomes	12 weeks	3
Hayes et al, Unpublished UK data 2007.	Solardomes	12 weeks	3
Hayes et al. 2011. Environmental Pollution 159:2420-2426.	Solardomes	20 weeks	2
Mills et al 2009. Global Change Biology 15:1522-1533.	Solardomes	20 weeks	2
Hayes et al unpublished data from 2007/2008 similar to Mills et al.,2009 (above)	Solardomes	20 weeks	2
Wagg et al 2012. Environmental Pollution: 165:91-95.	Solardomes	16 weeks	2
Hayes et al Unpublished UK data from 2011/2012.	Solardomes	12 weeks	3
Hayes et al. solardomes calcareous communities unpublished UK data from 2009/10	Solardomes	24 weeks	5
Hayes et al., 2012, Environmental Pollution 163:40-47.	Solardomes	24 weeks	5
Hayes et al Unpublished UK data from 2009/2010 using a similar set-up to Hayes et al 2012	Solardomes	24 weeks	3
Tonneijck et al., 2004. Environmental Pollution 131:205-213.	OTCs	15 weeks	4
Wyness et al 2011. Environmental Pollution 159:2493-2499	Solardomes	23 weeks	2

Table S2: Environmental responses and g_{max} of selected species, including the number of datapoints used to parameterise the DO_3SE model. Note that a standardised parameterisation was used for the flux-effect relationships presented.

Species	**No. of datapoints**	**gmax (ozone)**	**tmin**	**tmax**	**topt**	**VPD min**	**VPD max**	**SWP min**	**SWP max**	**light a**
Dactylis glomerata	880	262	12	32.6	24	3.2	1.2	-0.1	0	-0.01
Anthoxanthum odoratum	272	198								
Briza media	106	112								
Ranunculus acris	512	219	12	36	24	3.2	1.8	-0.12	-0.02	-0.005
Leontodon hispidus	546	408	6	29	21	3.2	1.2			-0.012
Campanula rotundifolia	317	219	9	41	25	3.2	1.2	-0.2	0	-0.006
Sanguisorba minor	355	259	12.5	34	25	3.2	2	-0.4	-0.05	-0.007
Sanguisorba officinalis	449	141	8	34	21	3.5	1.5	-1.5	0	-0.015
Leontodon autumnalis	115	112	11	33	23	3.2	1.2	-0.015	0	-0.07
Scabiosa columbaria	346	273	14	34	26	3.5	1.2	-0.3	-0.05	-0.007
Primula veris	423	95	8	38	23	3.5	1.7	-0.2	0	-0.015
Fritillaria meleagris	254	37	12	34	22	3.5	2	-0.25	0	-0.008
Potentilla erecta	411	134	10	35	25			-0.07	0	

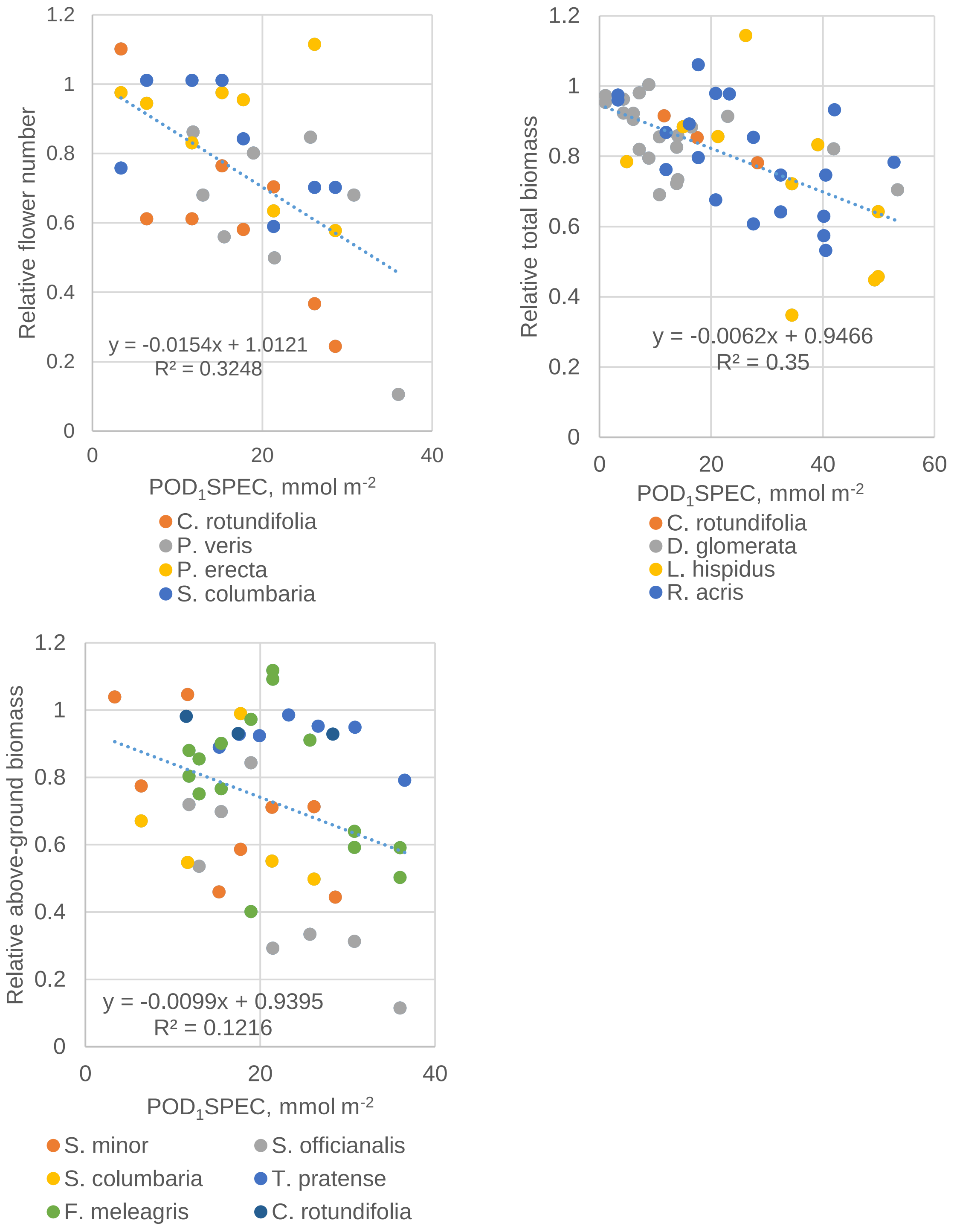

Figure S1: Relative total biomass, relative above-ground biomass and relative flower number related to POD_1SPEC, with the individual species used in the plots separately identified.