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Developing ozone critical levels for multi-species canopies of Mediterranean annual pastures[☆]

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ABSTRACT

Ozone (O_3) critical levels (CLe) are still poorly developed for herbaceous vegetation. They are currently based on single species responses which do not reflect the multi-species nature of semi-natural vegetation communities. Also, the potential effects of other factors like the nitrogen (N) input are not considered in their derivation, making their use uncertain under natural conditions.

Exposure- and dose-response relationships were derived from two open-top chamber experiments exposing a mixture of 6 representative annual Mediterranean pasture species growing in natural soil to 4 O_3 fumigation levels and 3 N inputs. The Deposition of O_3 and Stomatal Exchange model (DO_3SE) was modified to account for the multi-species nature of the canopy following a big-leaf approach. This new approach was used for estimating a multi-species phytotoxic O_3 dose ($POD_{y\text{-}MS}$). Response relationships were derived based on O_3 exposure (AOT40) and flux ($POD_{y\text{-}MS}$) indices.

The treatment effects were similar in the two seasons: O_3 reduced the aboveground biomass growth and N modulated this response. Gas exchange rates presented a high inter-specific variability and important inter-annual fluctuations as a result of varying growing conditions during the two years. The AOT40-based relationships were not statistically significant except when the highest N input was considered alone. In contrast, $POD_{y\text{-}MS}$ relationships were all significant but for the lowest N input level. The influence of the N input on the exposure- and dose-response relationships implies that N can modify the O_3 CLe. However, this is an aspect that has not been considered so far in the methodologies for establishing O_3 CLe. Averaging across N input levels, a multi-species O_3 CLe ($CLe_{f\text{-}MS}$) is proposed $POD_{1\text{-}MS} = 7.9$ mmol m^{-2}, accumulated over 1.5 month with a 95% confidence interval of (5.9, 9.8). Further efforts will be needed for comparing the $CLe_{f\text{-}MS}$ with current O_3 CLe_f based on single species responses.

1. Introduction

Tropospheric Ozone (O_3) is an important greenhouse gas that is also considered the most phytotoxic air pollutant, inducing negative effects on growth, productivity and yield quality of forests, crops and pastures (Ainsworth et al., 2012; Fuhrer, 2009).

Background O_3 concentrations have been raising since the Industrial Revolution in many parts of the Northern Hemisphere (Stevenson et al., 2006). Although the implementation of environmental policies in the last two decades has decreased peak O_3 concentrations (Klingberg et al., 2014), the air quality in Europe is still not acceptable for protecting the human health and the environment (EEA, 2014).

The Convention of Long-Range Transboundary Air Pollution (CLRTAP) of the United Nations Economic Commission for Europe (UNECE) is leading the process of setting O_3 critical levels (CLe) as policy target values for the protection of vegetation. Initially, O_3 CLe made use of exposure indices based on the atmospheric O_3 concentration (CLe_c) (Karenlampi and Skarby, 1996). More recently, O_3 stomatal uptake or flux-based CLe (CLe_f) have been developed in recognition that effects are more related with the amount of O_3 being absorbed by the leaves (Emberson et al., 2000a; Mills et al., 2011). CLe_f are used within the CLRTAP for performing O_3 risk

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assessments where the O_3 stomatal fluxes, also known as Phytotoxic O_3 Doses (POD_y), are computed by the Deposition of Ozone and Stomatal Exchange model (DO_3SE).

The current DO_3SE model configuration computes POD_y values for a big-leaf of a single species (Emberson et al., 2000a; CLRTAP, 2010). However, this represents a limitation when the model is used to assess the risk of O_3 effects on plant communities composed by different species like pastures. The community-level response to O_3 is the aggregated response of the component species and the existing interactions between them (Bassin et al., 2007; Gonzalez-Fernandez et al., 2008; Calvete-Sogo et al., 2016). Besides, pasture species often present a great range of stomatal conductance (g_{sto}) values (e.g. Gonzalez-Fernandez et al., 2010). Thus, the O_3 risk assessment methodology for pastures could be improved by using POD_y estimates that integrate the species composition of the pasture.

Mediterranean annual pastures are one the most valuable ecosystems in Southern Europe. In the Iberian Peninsula, annual pastures constitute the understory of Dehesas, a traditional agroforestry system (Montado in Portugal) covering nearly 20.000 km^2 (Diaz et al., 2007). Dehesas present a high vascular plant richness estimated at 230 species (Garcia del Barrio et al., 2014). This diversity is comparatively higher than other forest and agroforestry ecosystems from temperate climates (Díaz et al., 1999; Ojeda et al., 2000). Dehesas are located in areas frequently exceeding current European air quality limit values (EU Air Quality Directive, 2008/50/EC) for the protection of ecosystems. The particular climatic conditions of the Mediterranean area with high temperature and solar radiation and stable atmospheric conditions favour photochemical reactions that produce O_3 (Millan et al., 2000; Cristofanelli and Bonasoni, 2009). This results in some of the highest surface O_3 concentrations in Europe (EEA, 2014).

Ozone has been recognized as an important negative factor affecting the yield, reproductive capacity and species composition of annual pasture species (Bermejo et al., 2003; Gimeno et al., 2004a, b; Calvete-Sogo et al., 2014, 2016). These studies also showed how atmospheric nitrogen (N) deposition interacts with O_3, where N can mitigate the O_3-induced effects and O_3 reduces the efficiency of the N fertilization. Nonetheless, there is still the need to expand the experimental basis for evaluating the effect of O_3 and its interaction with N under close to field conditions and to increase the information available about the ecophysiology and gas exchange behavior of annual species for developing more robust CLe_f.

In this study, a new O_3 and N experiment with annual Mediterranean pastures following the same methodology than in Calvete-Sogo et al. (2014) is presented. The combined experimental results from the two seasons are analyzed through an improved O_3 flux model considering a multi-species approach and the influence of N on the response of the pasture to O_3. The inclusion of the specific O_3-sensitivity and gas exchange rates of the component species in this new approach will allow to propose more realistic CLe for Mediterranean annual pastures communities. The hypotheses were that higher CLe will be obtained with multiple species compared with individual sensitive species due the introduction of O_3-resistant species in the model and that nitrogen availability will modify the response to O_3. The main objectives of this work are: (1) to present a new experiment on the response of an annual community to O_3 and N; (2) to set a parametrization of the multiplicative algorithm of DO_3SE for g_{sto} modelling combining the six assayed species; (3) to estimate the POD_y for a multi-species canopy ($POD_{y\text{-}MS}$); (4) to develop exposure- and dose-response functions for yield considering the multi-species approach and the influence of N availability, and suggesting CLe_c and CLe_f for annual pastures based on this method.

2. Material and methods

2.1. Experimental design

In February 2011 and 2012 seeds were sown on the ground of 12 open-top chambers (OTC) located in central Spain (450 m.a.s.l.; 40°3′N, 4°26′W). Plants were exposed to four O_3 treatments from 7:00 to 15:00 GMT, seven days a week (3 OTC per O_3 treatment): charcoal filtered air (FA), non-filtered air (NFA) reproducing ambient levels, non-filtered air supplemented with 20 nl l^{-1} O_3 (NFA+) and non-filtered air supplemented with 40 nl l^{-1} O_3 (NFA++). Three ambient plots (AA) were also considered. Ozone was supplemented by a generator (Model 16, A2Z Ozone Systems Inc., USA) system fed with pure oxygen. A time sharing system registered the pollutants levels inside the OTCs and AA plots (O_3, SO_2 and NO_x) above the canopy (50 cm above the soil). All plots were divided in three sectors (1.4 m^2 each) following a split-plot design with three N treatments: N-low (soil N background), N-medium (N addition equivalent to 20 kg N ha^{-1}) and N-high (40 kg N ha^{-1}). To reach these integrated doses, N supplementation was applied every 2-weeks using a NH_4NO_3 solution.

Micro-meteorological conditions were continuously monitored inside the different chambers: air RH and temperature, photosynthetic active radiation (PAR), PAW (Plant Available Water, difference in water content between field capacity and permanent wilting point) and temperature at 10–15 cm depth. More details of the experimental design can be found in Calvete-Sogo et al. (2014).

2.2. Plant material and harvests

The simplified pasture community was composed of six representative species from Dehesa acidic annual grasslands. Three legumes (*Trifolium striatum, Trifolium cherleri, Ornithopus compressus*), two grasses (*Briza maxima, Cynosurus echinatus*) and one forb (in 2011 *Silene gallica;* in 2012 *Petrorhagia nanteuilii*) were sown by hand using a mixture of seeds on 11th and 27th February in 2011 and 2012 respectively. More details about the plant material preparation and sowing can be found elsewhere (Calvete-Sogo et al., 2014). Plant emergence was in 25th February 2011 and 9th March 2012 and considered as day 0 after emergence (DaE). Exposure to the different O_3 treatments started in April, 47 DaE (2011) and 57 DaE (2012). On the same date the first N fertilization dose was applied. The pasture was exposed to O_3 for 49 and 56 days in 2011 and 2012 respectively until the community reached its maximum development and productivity. Then the O_3 exposure system was switched off allowing plants to dry up and complete seed maturation, which happened in late May and early June in 2011 and 2012 respectively.

Aboveground biomass was harvested three times throughout the life span of the pasture in both years. The first harvest was done just before the start of O_3 treatments and N applications. The homogeneity of the species distribution between the sub-plots was tested at this harvest. The second harvest was collected after 39 (2011) and 38 (2012) days of O_3 exposure, at 84 DaE in both years, when the pasture reached its maximum biomass and blooming development; and the last harvest was done when plant biomass was completely dry and seeds had reached maturity, 116 DaE (2011) and 115 (2012). For each harvest, all plants within a 5 dm^2 sampling ring were cautiously collected in the central part of each treatment to avoid any border effect in the sub-plot. The biomass was sorted into species and immediately weighed to obtain the fresh weight. Afterwards, samples were dried to constant weight at 60 °C. The canopy yield was the sum of the dry weight of the different species expressed as biomass per square meter.