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Challenges, gaps and opportunities in investigating the interactions of ozone pollution and plant ecosystems

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Climate change and air pollution are interlinked and are a threat to plant ecosystems. Tropospheric ozone (O_3) impacts on plant ecosystems are of major concern globally, given the present distribution of O_3 pollution (Mills et al., 2018a) and the phytotoxicity of high O_3 levels (Paoletti, 2007). Ozone is an air pollutant formed in sunlight from photochemical reactions of its precursors such as nitrogen oxides and volatile organic compounds. While O_3 is a normal component of the troposphere, its background concentrations in the Northern Hemisphere have doubled since pre-industrial times (Vingarzan, 2004; Parrish et al., 2012; Cooper et al., 2014), with negative effects on human and plant health (Oksanen et al., 2013; WHO, 2013; Lelieveld et al., 2015; Lelieveld and Pöschl, 2017; Mills et al., 2018a). Ozone causes cellular damage in plants, inducing reduced stomatal control, lower CO_2 assimilation rates, and the occurrence of visible leaf injury (Fares et al., 2013; Jolivet et al., 2016; Ainsworth, 2017). These effects often accelerate senescence, diminish green leaf area and biomass, and reduce photosynthetic capacity (Jolivet et al., 2016; Ainsworth, 2017). Hence, O_3 pollution has large impacts on plant functioning, and, consequently on plant ecosystem productivity and services (Karnosky et al., 2007; Lindroth, 2010), as well as agricultural yields (Oksanen et al., 2013; Tian et al., 2016; Tai and Val Martin, 2017; Mills et al., 2018b).

Progress has been achieved by controlling the emission of O_3 precursors in some areas of the world, but much remains to be done (Lefohn et al., 2018). On 21-24 May 2018, an international conference was organized in Florence (Italy), enabling all experts studying the interactions between O_3 and plant ecosystems to meet and discuss the state of the art and the strategies for continuous improvements. The conference was co-organized by the International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) and the International Union of Forest Research Organizations (IUFRO) Research Group8.04.00 (former RG7.01.00) *Impacts of Air Pollution and Climate Change on Forest Ecosystems* including the three Working Parties on "*Genetic, biochemical and physiological processes*", "*Modelling and risk assessment*" and "*Ground-level O_3*". The ICP Vegetation is an international research programme investigating the impacts of air pollutants, including O_3, on crops and (semi-)natural vegetation, with a focus on impacts of pollutant mixtures (e.g. O_3 and nitrogen), consequences for biodiversity and the modifying influence of climate change on the impacts of air pollutants on vegetation (Harmens et al., 2015). The ICP Vegetation (https://icpvegetation.ceh.ac.uk/) reports to the Working Group on Effects (WGE) of the UNECE Convention on Long-Range Transboundary Air Pollution (LRTAP) (http://www.unece.org/env/lrtap/welcome.html). The IUFRO is the largest forest research network in the world (https://www.iufro.org/). The aim of RG 8.04.00 is to promote international cooperation, to encourage an interactive process among scientists, policy makers and representatives of local to regional governments and institutions, in order to share scientific knowledge and harmonize effective strategies aimed to reduce the risk for forests related to air pollution and climate change. Because of the recent establishment of the IUFRO working party on ground level O_3, a special focus is on the impacts of O_3 on forests.

The main themes of the conference were: 1. Monitoring, modelling and assessing the risk of O_3 damage to plant ecosystems. Proofs of the impacts of ambient O_3 on plant ecosystems are still elusive. New monitoring approaches and epidemiological studies are developing. Modelling of O_3 is becoming more and more sophisticated and of high resolution. Risk assessment is evaluating many different metrics for plant protection, with a focus in Europe on stomatal O_3 flux. 2. How plant ecosystems respond to O_3 exposure, including effects on forests, grasslands and consequences for food security. A main aim was to evaluate strategies for maximizing yield, productivity and other environmental services of plant ecosystems under O_3 stress. 3. How plant ecosystems affect O_3 concentrations in the atmosphere. Ozone deposition is strongly affected by the type of vegetation. Emission of biogenic volatile organic compounds is known to contribute to O_3 chemistry in the atmosphere. Mechanisms, seasonality and responses to O_3 singly and in combination with other environmental factors, as well as selection of appropriate green infrastructure for urban greening were discussed.

The conference participants discussed actual and emerging research challenges, knowledge gaps and opportunities in investigating the interactions of O_3 pollution and plant ecosystems. From the oral and poster presentations, 24 papers were peer-reviewed and published in a dedicated special issue in Science of the Total Environment, available at http://www.sciencedirect.com/science/journal/00489697/vsi/10Q8QW4D8R7. The published special issue provides a source of new knowledge regarding status, trends and impacts of O_3 pollution as well as plant physiological mechanisms and ecological effects under O_3 singly or combined with other environmental factors. Some of the main findings of the published papers are summarized herein, by grouping the papers into four categories (note: some of the papers provide new and important insights that fall within more than one categories, but are discussed only in one category for presentation purposes; the reader may refer to the original articles for further reading):

1) Three papers deal with air pollution status, trends, and real-world impacts on forest trees, and one paper deals with dose-response models used for the evaluation of O_3 effects and derivation of critical levels. Zeng et al. (2019) illustrate that while the levels of SO_2, NOx and particulate matter (PM) have been reduced over the last decade, the levels of O_3 are increasing in China. They also found that the values of the average 90^{th} percentile of daily maximum 8-hour average O_3 concentration (90th MDA8), annual mean of the weekly average O3 concentrations from 09:00 to 16:00 (M7), and cumulative exposure to hourly O_3 concentrations exceeding 40 ppb (AOT40) showed an increasing trend in 31 capital cities over the time period 2013-2017. The work by Zeng et al. (2019) also suggests that China's air pollution is now NOx and O_3-dominated, highlighting that O_3 will remain a major air pollutant threatening plants in the many years to come. Araminienė et al. (2019), based on data from 2001 onward, found that the annual mean O_3 concentration (- 0.28 ppb per decade) and AOT40 (- 2,540 ppb h per decade) decreased, whereas the Phytotoxic O_3 Dose over a threshold of 0 nmol m^{-2} s^{-1} (POD_0) increased (0.4 mmol m^{-2} per decade) in Lithuania. AOT40 and POD_0 were correlated with crown defoliation and visible foliar injury, respectively, in ICP-Forests plots; however, the visible injury was negligible in terms of magnitude. Hůnová et al. (2019) mapped AOT40 and N deposition in Czech forests over the years 2000–2015, and found higher N deposition in northern areas while southern areas had higher O_3 exposures. Interestingly, areas with a potential risk from simultaneously high O_3 exposure and N deposition represented only less than 5% of the total forested area.

2) Seven papers deal with dose (or exposure)-response relationship for evaluating O_3 effects on plants and risk assessment. Agathokleous et al. (2019a) evaluated published literatures on O_3 effects on plants as well as the most recent developments in toxicological dose-response research to assess the biological relevance of different dose-response models as to their biological suitability for risk assessment. Their study documents a wide occurrence of O_3-induced hormesis in plants, which results from the activation of the *adaptive response* by low O_3 exposures/doses. Agathokleous et al. (2019a) suggest that the AOT40 metric is biologically irrelevant, and thresholds in the metrics should not be used, when assessing dose-response relationships to derive toxicological estimates. A further paper by Dusart et al. (2019) is an integrated experiment that analyzes biological mechanisms of plant response to O_3, and suggests that both linear threshold and

hormetic models can be observed in biological response indicators (e.g. defense- or repair-related) when using POD_0 as O_3 metric; this study also sheds light on detoxification mechanisms associated with the Halliwell-Asada-Foyer cycle and can feed risk assessment evaluations in the future, with a perspective to account for detoxification and repair processes that are currently excluded. By incorporating O_3 (120 ppb, 17 days) and mild water deficit stress singly or in combination, Dusart et al. (2019) also suggest that antagonistic effects between water deficit stress and O_3 can modify the slope of the dose-response relationship and the magnitude of the response in the hormetic model. Pellegrini et al. (2019) cultivated three oak species (*Quercus ilex*, *Q. pubescens* and *Q. robur*) under single and combined effects of O_3 (1.0, 1.2 and 1.4 times the ambient O_3 concentration) and water availability (100, 80 and 42% of field capacity). They observed that both O_3 and drought enhanced carotenoids, decreased flavonoids and prevented the peroxidation by free radicals in *Q. ilex* and *Q. pubescens*, but induced a partial readjustment of the phenylpropanoid pathway and cell structure damage in *Q. robur*, suggesting that *Q. robur* is less tolerant than *Q. ilex* and *Q. pubescens*. They further assessed the POD_0-malondialdehyde response relationships and proposed that accelerated leaf senescence can be assessed in deciduous oak species using the POD approach. Shang et al. (2019), after exposing two clones of poplar to O_3, evaluated exposure-response relationships using AOT40 as O_3 metric and leaf mass per area, photosynthetic N-use efficiency and leaf N concentration per area or per mass as response indicators. They demonstrated that the slope of the exposure-response relationship differed between the two clones when N concentration was expressed per leaf area but not when N concentration was expressed per leaf mass. This study provides important insights for selecting response indicators. Dai et al. (2019) evaluated experimentally whether N load affects the O_3 stomatal flux-response relationship for birch saplings biomass, and found that O_3 dose-response relationships for biomass were not affected by N load. This study suggests a need for further long-term studies and with different species to confirm whether the nature of the O_3 dose-response relationships and the thereby toxicological estimates are affected by N load. Pleijel et al. (2019) utilized published data to assess O_3 impacts on wheat (*Triticum aestivum*) grain yield in Europe, Asia and North America using dose-response analysis. They concluded that, on average, the response was lower for the older North American experiments but the grain mass and harvest index responded similarly for Europe, Asia and North America. This study also highlights the importance of the response indicator (plant trait) for dose-response relationships and risk assessment. Feng et al. (2019) conducted a meta-analysis on O_3 effects on poplars, and found that current ambient O_3 levels may reduce photosynthesis by 33% and total plant biomass by 4%, and that high O_3 (mean=88 ppb) reduces isoprene emission rate by 34%. Furthermore, exposure-response relationships of photosynthesis, leaf chlorophyll concentrations and total biomass of poplars using global data were provided for the first time. This study provides important information for air pollution feedbacks due to O_3 as well as for improving O_3 risk assessment.

3) Seven papers report on the interactive effects of O_3 with other environmental factors, in particular salinity, fertilization and soil water availability. Calzone et al. (2019) studied single and combined effects of O_3 and salinity on pomegranate plants (*Punica granatum* cv. Dente di cavallo) for three months and found that leaf antioxidative adjustments in the presence of both elevated O_3 (AOT40=58.7 ppm h) and salinity were insufficient to ameliorate the O_3-induced oxidative stress. Sugai et al. (2019) assessed the effects of N loading and O_3 on Japanese larch (*L. kaempferi*) and its hybrid F_1 (*L. gmelinii* var. *japonica* × *L. kaempferi*) over two growing seasons (three months of exposure per growing season). They found that N loading (50 kg $(NH_4)_2SO_4$ ha^{-1} yr^{-1}) reduced the negative effects of O_3 on Japanese larch but did not reduce the negative effects of elevated O_3 (average monthly AOT40= 7.0 ppm h) on growth and photosynthetic capacity of hybrid larch. Higher growth response to N load contributed to more severe O_3 effects in hybrid larch, and leaf N/P ratio seemed to have an important role in O_3 and N load responses. In another study, Podda et al. (2019) exposed an O_3-susceptible poplar clone to single or combined effects of O_3 (ambient, 1.5 × ambient and 2.0 × ambient), soil N (0 and 80 kg ha^{-1} yr^{-1}) and P load (P; 0, 40 and 80 ha^{-1} yr^{-1}) for five months. O_3 induced multiple stress signals, independently of the concentration. N and P fertilization restricted the accumulation of reactive oxygen species and enhanced membrane stability but only in ambient O_3 (14.4 ppm h) and 1.5 × ambient O_3 (43.8 ppm h); N and P fertilization could not mitigate the effects of 2.0 × ambient O_3 exposure (71.1 ppm h). Agathokleous et al. (2019b) treated cauliflower (*Brassica oleracea*) with O_3 (ambient≈20 ppb, elevated≈55 ppb) and/or N loading (0 and 50 kg $(NH_4)_2SO_4$ ha^{-1} yr^{-1}) for about one month in an open-field experiment. They found that N availability but not O_3 drove plant-herbivore interactions, through enhanced leaf N content. They followed this up with laboratory assays where polyphagous larvae (Eri silkmoth, *Samia ricini*) could feed on leaf tissues from either each experimental condition separately (no-choice) or all the experimental conditions together (choice). The field observations for preference toward N-treated leaves were confirmed by the choice assays; however, the no-choice assays also showed that larval body mass growth was inhibited when larvae fed on leaf tissues from elevated O_3 or high N load. Mrak et al. (2019) studied responses of roots and ectomycorrhizae communities in three oak species (*Q. ilex*, *Q. pubescens* and *Q. robur*) subjected to O_3 (1.0 and 1.4 times the ambient O_3 concentration) and/or water availability (100 and 10% of field capacity) for about 150 days (see also Pellegrini et al., 2019), and revealed greater effects of O_3 when plants were well-watered, although the effects were complex, species-specific and root-trait

specific. Likewise, Li et al. (2019) exposed a poplar clone to charcoal-filtered air and ambient air enriched with 40 ppb of O_3 as well as to different irrigation regimes and soil N loads (50 kg N ha^{-1} yr^{-1}) for 104 days, and found that elevated O_3 (AOT40=41.6 ppm h) reduced total plant biomass but not when irrigation and soil N were limited. Finally, Landi et al. (2019) subjected two deciduous oak species (*Q. cerris* and *Q. pubescens*) to either full irrigation or 15-day water withholding (20% of daily evapotranspiration) and, then, to either filtered air or 200 ppb O_3 for 5 h. They found that *Q. cerris* had a higher capacity to propagate the wave of O_3-induced reactive oxygen species than *Q. pubescens*, even in water-limiting conditions, thus, its PSII function was better protected when the episodic O_3 pulse occurred. *Q. pubescens* lost its ability to cope with O_3 when subjected to water withholding; thus, it was more susceptible to the episodic O_3 pulse than *Q. cerris* in water-limiting conditions.

4) Seven papers examine mechanisms of O_3 effects on plants and plant-interacting microbes and insects. Fernandes et al. (2019) demonstrated that the liana species *Passiflora edulis* Sims was tolerant to O_3 exposures elevated up to twice the ambient concentration for about three months, and its tolerance was related to enhanced non-enzymatic antioxidants (ascorbic acid, carotenoids, glutathione and flavonoids), hyperplasia and hypertrophy of the mesophyll cells, and other morphological acclimation responses. Gandin et al. (2019) exposed ten Euramerican poplar genotypes (*Populus deltoides* × *nigra*) to 120 ppb of O_3 for 3 weeks to shed light on the relative contribution of different biological mechanisms to O_3 tolerance. They found that growth and productivity can be maintained by protecting photosynthetic capacity through ascorbate peroxidase and ascorbate regeneration through monodehydroascorbate reductase, which were the major determinants of O_3 tolerance. Yadav et al. (2019), after exposing early and late sown wheat cultivars to ambient and elevated (ambient + 20 ppb) O_3 levels for one growing season, concluded that cultivars that were sown early outperformed cultivars that were sown late in their defense response due to higher induction of enzymatic and non-enzymatic antioxidants. However, this study also suggests that cultivars that were sown early may be more susceptible to elevated O_3 because of the extra metabolic cost that non-enzymatic defense mechanisms require compared to enzymatic defense. Marchica et al. (2019) conducted a sequence genome analysis of common sage (*Salvia officinalis*) exposed to 200 ppb O_3 and found that the genes *WRKY4*, *WRKY5*, *WRKY11* and *WRKY46* were up-regulated after 2 and 5 hours of O_3 exposure. These results suggest that WRKYs were important for regulating signaling mechanisms during the initial response of plants to O_3. These studies also provide new insights into the role of ethylene, salicylic acid and jasmonic acid in O_3 defense mechanisms (Landi et al. 2019; Marchica et al. 2019), but also highlight the complexity of the signaling network in plants exposed to multiple stresses. Xu et al. (2019) cultivated an O_3-

sensitive hybrid poplar clone (*Populus deltoides* cv. 55/56 × *P. deltoides* cv. Imperial) in charcoal-filtered ambient air or elevated O_3 (40 ppb above ambient concentration), for about 100 days, and showed that inhibition of light-saturated net photosynthesis by O_3 was associated more with decreased mesophyll conductance, little with carboxylation and not with stomatal conductance. Although the magnitude of the responses varied with time and leaves, this study suggests that mesophyll conductance is a key determinant of photosynthesis under elevated O_3. Wang et al. (2019) exposed a temperate grassland to O_3 for 4 years (4-years average AOT40=1.3, 14.0 or 26.4 ppm h for three O_3 treatments, respectively). Their results show that total soil carbon and *β*-glucosidase activity were decreased by elevated O_3; however, microbial activities were not affected significantly by O_3 or its interaction with aggregate size. Nonetheless, the size and activity of the microbial community were altered by elevated O_3. Zhang et al. (2019) studied bacterial communities in a rotation paddy system with summer rice (*Oryza sativa*) and winter wheat (*T. aestivum*) exposed to ambient or elevated O_3 for 4 growing seasons (4-year average daily mean ≈ 37 or 48 ppb, respectively). With the elevation of O_3 levels, bacterial alpha diversities were stimulated through a survival strategy in the presence of limited resources, which resulted in the instability of the community, and the temporal turnover of the bacterial community composition was decelerated as a result of plant-derived deterministic processes. A collection of four studies published in this special issue provides further novel insights on O_3 indirect effects on microbes and insects (Agathokleous et al., 2019b; Mrak et al., 2019; Wang et al., 2019; Zhang et al., 2019), suggesting that elevated O_3 may pose an indirect threat to trophic interactions. The published papers provide a platform upon which future developments can be based.

Thanks to the broad participation of experts from different countries and scientific fields, the conference was a fundamental moment to define the state-of-the-art of the challenging interactions between O_3 and plant ecosystems. More field-based evidence of O_3 impacts (monitoring and experimental data in both developing and developed regions, and use of epidemiological data) and O_3 interactions with other stressors related to a changing climate. Flux-based metrics are the most biologically relevant indicators for O_3 risk assessments and must be proposed as standards for ecosystem protection. A necessity emerged to improve the research network and establish further science policy frameworks, especially in developing regions.

The following key issues were identified of major interest at present: a) General updates on O_3 trends in different countries and ecosystems; b) Regional risk assessment of ambient O_3; c) New developments in modeling of O_3 deposition for forest trees and crops; d) Multi-scale monitoring approaches; e) Big data validation and analyses (e.g. TOAR, GAW database); f) Active monitoring of hourly O_3 concentrations and phytotoxic O_3 dose calculations; g) Mechanisms of O_3 impacts and detoxification (molecular, physiological, and stomata); h) Latest results from multifactorial studies, the effect of O_3 on plants in combination with other biotic and abiotic stressors; i) Impacts of O_3 on below-ground processes and nutrient cycling; j) O_3 impacts on non-woody (semi-)natural vegetation, e.g. grasslands; k) Joint use of O_3 research facilities e.g. ozone FACEs; l) Impacts of O_3 on vegetation in urban areas and role of vegetation in cleaning air in cities.

Future goals include the incorporation of O_3 impacts in crop and tree growth models, in modelling future impacts in the context of a changing climate (e.g. drought, warming and elevated CO_2) and developing epidemiologically-based O_3 critical levels for ecosystem protection against O_3, as recommended by the LRTAP Convention and for application in the EU National Emission Ceilings Directive (NECD) (EU, 2016; European Environment Agency, 2018). Non-linear dose-response relationships should be considered, especially for biological response indicators if detoxification capacity is incorporated in the derivation of critical levels in the future. As a future activity, scientists should work for a better definition of O_3 impacts on the complexity of ecosystems services, as well as for the investigation of "management" solutions for crops, forests and semi-natural ecosystems. New opportunities in the field are related to studies focusing on the socioeconomic and environmental evaluation of O_3 impacts on crops and terrestrial ecosystems. Ozone experts can also contribute to the greening of cities to improve air quality and human well-being, defining the most suitable species differentiated for geographical areas. Finally, the conference warrant that the community should work for improving knowledge transfer to stakeholders, in particular policy makers, regarding the O_3-plant ecosystem interactions. These goals will be discussed, in light of new evidence, at the next international conference entitled "*Air Pollution Threats to Plant Ecosystems*" that will be held on 4-8 May, 2020, in Paphos, Cyprus (http://www.ozoneandplants2020.com/).

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