Sustainable Horizons 2 (2022) 100018

Contents lists available at ScienceDirect

Sustainable Horizons

journal homepage: www.elsevier.com/locate/horiz

Towards long-term sustainability of stomatal ozone flux monitoring at forest sites

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ARTICLE INFO

Keywords :
Tropospheric O_3
Stomatal ozone uptake
POD
Forest Monitoring
Visible injury
Forest protection

ABSTRACT

Stomatal O_3 flux accumulated over the growing season (or phytotoxic ozone dose above a y threshold of uptake, PODy) is nowadays considered as the best biologically based metric to assess O_3 injury to vegetation and establishing critical levels (CLs). So far, CLs have used biomass losses as forest-health indicator in experimental research. Ozone-induced biomass losses of adult forests, however, are difficult to assess. We stress a need to reconcile present CLs and dose-response functions to estimate O_3-induced biomass losses. In fact, a clear discrepancy emerges when comparing CL exceedances calculated from WRF-Chem results and biomass losses estimated based on the presently available dose-response functions for different forest types. We synthesize recent evidence that visible foliar O_3 injury (VFI) recorded annually in large-scale forest surveys may be used as forest-health indicator and coupled with active O_3 monitoring in setting epidemiology-based CLs for forest protection. Bridging experimental and monitoring research is needed for validation and parameterization of field results and models, with the O_3 free-air controlled exposure (FACE) facilities as a reliable experimental tool. In fact, VFI is well correlated to O_3-induced biomass loss at O_3 FACE experiments, and POD1 and O_3 tolerance (when characterized as leaf mass per area (LMA) directly affect VFI under field conditions. POD1 shows a much stronger causality than the O_3 exposure index AOT40 in affecting VFI. Accurate calculation of PODy at field sites is now possible, thanks to recent technological advances that support low-cost and reliable monitoring of PODy at forest sites and thus ensure long-term sustainability of such laborious monitoring. We summarise evidence that active O_3 monitoring is a cost-effective approach for estimating PODy and its economic effects on forests, as the initial investment is compensated in few years by less travels to the field sites than passive O_3 monitoring. These results respond to raising legislative interest into PODy, and support long-term sustainability of PODy monitoring at forest sites.

1. Introduction: The ozone threat to forests

Climate change and air pollution are interlinked challenges, since they affect each other via complex interactions both in the atmosphere and with the ecosystems (Bytnerowicz et al., 2007). Tropospheric ozone (O_3) is the third most important greenhouse gas in terms of radiative forcing (Chen et al., 2007) and one of the main air pollutants regulated in the European Union (European Union (EU) Directive, 2008; European Parliament and Council DIRECTIVE (EU)., 2016), USA (United Stated Federal Register., 2014; EPA - U.S, 2022), and other world regions (WHO, 2008; Chinese Ministry of Environmental Protection, 2012). Tropospheric O_3 is not emitted directly, but forms when sunlight triggers reactions between natural and anthropogenic emissions, known as O_3 precursors, such as methane, carbon monoxide, volatile organic compounds, nitrogen oxides (NOx) (Kondratyev and Varotsos, 2001). The anthropogenic O_3 precursor emissions decreased in North America and Europe since the 1990s, while eastern Asian emissions have slightly decreased since 2010 (Zheng et al., 2018). Since the 1990s, a reduction in O_3 mean concentrations was observed in rural areas worldwide, in particular from 2005 onwards, with a reduction of 0.24 ppb year^{-1} in North America and 0.41 ppb year^{-1} in Europe (Sicard, 2021). In East Asia, an increase of 0.21 ppb year^{-1} was reported at rural stations between 2000 and 2010, while slight decreases were recently reported at rural stations nearby Beijing in 2007-2018 (Xu et al., 2020). As O_3 concentrations are highly dependent on environmental

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https://doi.org/10.1016/j.horiz.2022.100018
Received 20 May 2022; Received in revised form 21 June 2022; Accepted 22 June 2022

conditions, including air temperature, they are expected to increase with climate change (Meleux et al., 2007). At regional background stations, the baseline O_3 levels significantly increased in the Northern Hemisphere by on average 0.15 ppb year^{-1} over the last three decades, which was attributed to climate change (e.g., higher air temperature and methane emissions, changing lightning and NO_x emissions), long-range transport, and increased precursors emissions in Asia (Lefohn and Cooper, 2015). Therefore, the issue of non-attainment of the target value for O_3 persists in Europe, North America and Asia (Sicard et al., 2017).

Tropospheric O_3 is a highly reactive, oxidative gas associated with adverse health outcomes, especially on respiratory and cardiovascular systems, including mortality and morbidity (Nuvolone et al., 2018). In addition, O_3 seriously threatens plants by altering their biochemistry and physiology (Paoletti, 2007), thus impairing photosynthetic carbon assimilation (Gao et al., 2017) and stomatal control (Hoshika et al., 2018), accelerating leaf senescence (Podda et al., 2019), reducing biomass and carbon sequestration (Carriero et al., 2015), producing visible foliar injury (VFI) (Sicard et al., 2020; Moura et al., 2021), and altering biodiversity (Agathokleous et al., 2020). Hence, O_3 pollution has large impacts on plant functioning, and consequently on ecosystem services (Paoletti et al., 2010).

To assess the potential O_3 risk to vegetation, different metrics are available (Paoletti and Manning, 2007; Lefohn et al., 2018). The European standard is AOT40 (European Union, 2008European Union (EU) Directive, 2008), an index based on exposure, i.e., how much O_3 is in the air, and does not consider the fact that O_3 is harmful only when absorbed inside the leaves through the stomata (Paoletti and Manning, 2007). To overcome this shortcoming, a new metric was proposed, i.e., the phytotoxic ozone dose (PODy), defined as the accumulated O_3 flux entering the leaves via the stomata, over a detoxification threshold y (Emberson et al., 2000; Mills et al., 2011). Thanks to monitoring and experimental studies, it was found that exceedances of the AOT40 critical levels may not match with the O_3 effects on forest-health indicators (Sicard et al., 2016; Feng et al., 2019); therefore, PODy is considered as a better standard for the protection of forests against O_3 and is included into the revised European Community directive regulating the national emission ceilings and the monitoring of pollutant impacts on ecosystems (European Parliament and Council DIRECTIVE (EU)., 2016). Article 9 of this Directive states that “Member States shall ensure the monitoring of negative impacts of air pollution upon ecosystems through a cost-effective and risk-based approach, based on a network of monitoring sites” (de Marco and Sicard, 2019). Consequently, a renewed interest in monitoring stomatal O_3 flux at forest sites is arising.

Forest monitoring is a key step in the sustainable management and protection of forests from the combined threats due to air pollution and climate change (Silaghi and Badea, 2012), and is carried out within the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) operating under the UNECE (United Nations Economic Commission for Europe) Convention on Long-range Transboundary Air Pollution (CLRTAP) (Ferretti, 2013). Epidemiology compares large-scale biological responses with environmental data monitored in the field and helps establishing the best metrics and PODy-based critical levels (CLs) for forest protection against O_3 injury (Braun et al., 2017). In addition, monitoring air quality at forest sites together with forest characteristics, e.g., indicators of forest health, allows knowing the pollutant concentrations in the atmosphere; quantifying the adverse effects to forest trees and forest ecosystems; evaluating the effectiveness of the adopted legislative precautions; and ultimately ensuring the sustainability of national and trans-national forest monitoring programs.

The main aim of this paper is to review key findings emerging from recent experimental and monitoring research on the significance of PODy for forest health, with a focus on results from realistic field conditions, and to derive prospects for sustainable monitoring and research directions.

2. Stomatal ozone flux monitoring at forest sites

At forest sites, tropospheric O_3 can be monitored by either active monitors, that are continuously operating, mechanical, real-time instruments, or passive sensors, that are cumulative, total-exposure samplers (Bytnerowicza et al., 2002; Hůnová et al., 2003). The passive approach has been used since 2000 in Europe, e.g., at the Level II sites of the ICP Forests network (Calatayud and Schaub, 2013), while the active system is used at some ICP Forests sites (Schaub et al., 2020). Passive samplers are characterized by uncertainties (Krupa and Legge, 2000) and low temporal resolution, from one week to one month (Cox, 2003), while calculation of PODy and AOT40 requires hourly data. This implies the need to apply functions to estimate hourly concentrations from passive monitors (Loibl et al., 1994; Loibl et al., 2004; Krupa, 2001; Tuovinen, 2002; Calatayud et al., 2016), even though results may be dubious in inhomogeneous territories (de Marco et al., 2014). The continuous improvement of solar panel and active O_3 monitor technologies nowadays support active monitoring of PODy at forest sites. While no centralized calculation of PODy based on meteorological, soil water content (SWC) and O_3 measurements is performed in the framework of ICP Forests, individual countries use those data to calculate different O_3 metrics (including PODy) (Araminienė et al., 2019; Eghdami et al., 2022). No centralized calculation of PODy is operative because hourly climate and O_3 data are optional for ICP Forests countries, thus there is no adequate and complete database necessary for applying the DO3SE model, i.e., the model recommended for calculation of PODy (CLRTAP Mapping Critical Levels for Vegetation, 2017). In detail, PODy (mmol m^{-2}) is calculated, as:

$$\mathrm{PODy} = \int \max\left(\mathrm{F_{st}} - \mathrm{y}, 0\right) \times \mathrm{dt} \tag{1}$$

$$\mathrm{F_{st}} = \mathrm{CO_3} * \mathrm{g_s} * \mathrm{rc}/\left(\mathrm{rb} + \mathrm{rc}\right) \tag{2}$$

where F_{st} is the hourly mean O_3 flux through stomata (nmol O_3 m^{-2} s^{-1}), y is a flux threshold (nmol O_3 m^{-2} s^{-1}), dt is 1 h, CO_3 is the hourly O_3 concentration (ppb); rc and rb are the leaf surface and quasi-laminar resistances; and g_s is the hourly value of stomatal conductance to O_3 estimated by the DO3SE model. The hourly values measured at 2 m above ground level (a.g.l.) are extrapolated up to the actual tree crown height. PODy is accumulated over the period recommended by the EC air quality Directive (i.e., 1st April to 30th September; 8-20 CET).

The European LIFE programme funded a specific project (MOTTLES, LIFE15 ENV/IT/000183) for equipping a set of forest sites belonging to existing networks (ICP Forests in Italy and Romania, MERA in France) with new instrumentation in order to measure the field data needed for PODy quantification. Importantly, the approach is based on active monitoring of O_3 concentrations and meteorological parameters. In addition, forest-health indicators of O_3 impacts on vegetation, i.e., visible foliar O_3 injury and crown conditions, are surveyed annually. A description of the MOTTLES methodology and sites is in Paoletti et al. (Paoletti et al., 2019). Within MOTTLES, y thresholds of 0, 1 and 3 nmol O_3 m^{-2} PLA s^{-1} are evaluated for correlation with forest-health indicators. Most of the key findings summarized below are based on MOTTLES data.

3. Key findings

3.1. Visible foliar ozone injury can be used to set critical levels for real-world forest protection

The CL approach was developed within the UNECE CLRTAP for assessing the risk of air pollution impacts to ecosystems and was applied for emission reduction strategies under the 1999 Protocol to Abate Acidification, Eutrophication and Ground-level O_3 Critical levels are defined as the “concentration, cumulative exposure or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge". Since O_3 background concentrations are increasing, it is important to define appropriate and realistic CLs, representative of actual field conditions, in order to a) protect vegetation; b) improve understanding and monitoring of O_3 effects on ecosystems; c) scientifically assess the effectiveness of air pollution control strategies and d) undertake measures for abatement of O_3 precursors emissions (Sicard et al., 2016; de Marco and Sicard, 2019; Paoletti et al., 2019; de Marco et al., 2019). Following the revision of the European National Emission Ceiling Directive in 2016, consistent PODy-based CLs for forest protection against O_3 damage are of great interest (de Marco et al., 2019). To date, most experiments to establish biologically relevant plant responses to O_3, such as VFI, have been performed on seedlings under growth-chamber conditions that are not representative of actual field conditions, so that the results may not help developing realistic standards. Indeed, a standard for forest protection is biologically relevant when it translates into real-world forest impacts.

We expanded the dataset in Paoletti et al. (Paoletti et al., 2019) and Sicard et al. (Sicard et al., 2020) to the period 2017-2021 (details on the methodology are in these two papers), and correlated PODy to real-world impacts in terms of different forest-health indicators, namely VFI, crown defoliation, and radial growth for the 17 MOTTLES forest sites (Fig. 1). POD1 increased with increasing VFI, while AOT40 increased with increasing crown defoliation and decreased with decreasing radial growth and VFI. It is likely that the effects of AOT40 are affected by soil water availability (Anav et al., 2018). Overall, crown defoliation and radial growth are specific indicators as they respond to the many different biotic and abiotic co-factors that co-occur in a forest, including nutrients and water availability, climatic conditions, pests, and site characteristics, while VFI is specific of O_3 (Sicard et al., 2016). Based on these monitoring results, we recommended VFI as forest-health indicator and POD1 as O_3 metric.

Visible foliar O_3 injury in the forest may be monitored both within the forest plot (ITP) and along the Light-Exposed Sampling Site (LESS), i.e., a light–exposed forest edge close to the station where meteorological and O_3 concentration data are recorded (radius < 500 m, according to Schaub et al. (Schaub et al., 2016)). LESS is a non-destructive, less complex, and less time-consuming approach compared to ITP for monitoring VFI in the long term (Sicard et al., 2021). By finding significant correlations with POD1 at 9 forest sites in Italy in 2017-2019, (Sicard et al., 2021) suggested that the frequency of symptomatic plant species within a LESS is a suitable and effective forest-health indicator of O_3 phytotoxicity in forest monitoring. When we expanded the dataset (Fig. 1), however, only VFI of the dominant species in the plot (VIITP) was significantly correlated with POD1, suggesting that longer-term results are still needed to clarify the best approach to monitor VFI.

By reanalyzing MOTTLES data published by Sicard et al. (Sicard et al., 2020), we applied structural equation modeling (SEM) to investigate whether AOT40 or POD1 were better related to the VFI values recorded at Italian field sites (Fig. 2). In the case of AOT40, two direct factors (i.e., tolerance [characterized by leaf mass per area (LMA) (Feng et al., 2018) and AOT40 [characterized by AOT40 at each forest site]) resulted to cause VFI (Fig. 1). The analysis also indicated that both air temperature and precipitation negatively influenced the values of LMA, which agrees with the finding in a meta-analytic review by Poorter et al. (Poorter et al., 2009). POD1 was strongly affected by drought (Fig. 2B; SWC vs. POD1, path coefficient: 0.89, $p < 0.001$) as previously reported in manipulative experiments (Hoshika et al., 2018); in addition, POD1 and O_3 tolerance directly affected VFI. In the relationship with VFI, a much stronger causality was found for POD1 (Fig. 2b, path coefficient: 1.17, $p < 0.001$) than for the O_3 exposure index AOT40 (Fig. 2a, path coefficient: -0.17, $p = 0.662$). This reanalysis confirms that POD1 is better than AOT40 as it is closely related to VFI observed in real-forest monitoring.

In previous studies, CLs were derived from experiments under controlled conditions, not representative of field conditions (e.g., (Matoušková et al., 2010; Gonzalez-Fernandez et al., 2013), and by using biomass loss as plant health indicator for a reduction of 2% (Norway spruce) or 4% (beech and birch) in the annual growth of young trees under experimental conditions (e.g., (CLRTAP Mapping Critical Levels for Vegetation, 2017, Karlsson et al., 2006; Calatayud et al., 2011; Büker et al., 2015). In some short-term experiments, the presence of VFI did not always coincide with measurable biomass losses due to O_3 (Chappelka and Samuelson, 1998). By reanalyzing data from realistic ozone FACE experiments carried out over entire growing seasons, however, we found a clear relationship between total biomass reduction and VFI (Fig. 3), suggesting that occurrence of VFI in the field may be successfully used as a proxy of biomass losses in setting epidemiology-based CLs.

3.2. *Legislative reference periods and low y thresholds are recommended for calculating PODy*

For both PODy and AOT40, different reference periods are recommended by the EU legislation (European Union (EU) Directive, 2008) or CLTRAP bodies (CLRTAP Mapping Critical Levels for Vegetation, 2017) as accumulation windows. With an aim of clarifying which reference period is better suited for monitoring O_3 injury in real–world forests, we accumulated PODy and AOT40 over the period mentioned in the EC Directive 2008/50/EC (3European Union (EU) Directive, 2008), i.e. April to September, the period recommended by CLRTAP (CLRTAP Mapping Critical Levels for Vegetation, 2017), i.e. year–long for Mediterranean evergreen species or April to September for the other species during the daylight hours with global radiation higher than 50 W m^{-2}, and two periods proposed by the MOTTLES project, i.e. the actual growing season of the dominant tree species in the plot (based on in-field phenological observations of start and end of the growing season) and from the actual start of growing season to the date of the forest-health survey at a plot (Paoletti et al., 2019). Fig. 1 shows that all POD1s well correlated each other, thus suggesting that any reference period may be used. However, significant Spearman correlations between PODy and VIITP were found only for the periods recommended by the EU legislation and CLTRAP, thus suggesting that the present legislative accumulation periods are biologically relevant and have to be recommended for use.

Although CLRTAP (CLRTAP Mapping Critical Levels for Vegetation, 2017) recommends 1 mmol m^{-2} s^{-1} as y threshold for establishing the CLs for forest trees, many studies have focused on determining the most appropriate thresholds for different forest species and plant functional types (Hoshika et al., 2018; Büker et al., 2015). Usually, the best threshold is selected based on statistical analysis i.e. the confidence intervals of the dose-response function must include the Y-intercept = 1 and the highest R^2 value from the equations that were included in the previous criterion must be chosen (Hoshika et al., 2018, Büker et al., 2015). However, the risk is to produce a plethora of slightly different y thresholds for physiognomically-similar plant species, which may complicate risk assessment on regional and global scale. We selected three distinct y thresholds, i.e. 0, 1 and 3 mmol m^{-2} s^{-1}, and found that PODy was significantly correlated with VIITP when the lower thresholds 0 and 1 mmol m^{-2} s^{-1} were used (Fig. 1). A close match of POD1 and POD0 performances (Anav et al., 2019) and a better performance of lower y thresholds (Sicard et al., 2016; Araminienė et al., 2019; de Marco et al., 2015; Hoshika et al., 2020); Fig. 4 were reported in previous modelling or experimental studies, and overall support the CLRTAP (CLRTAP Mapping Critical Levels for Vegetation, 2017) decision of proposing POD1 as unifying metric for all forest species.

In the forest, upscaling stomatal O_3 fluxes typically estimated for sunlit leaves at the top of the canopy to the entire canopy is often a challenge. In order to solve such an issue, multi-layer canopy models may help. Such models combine algorithms of penetration of light with algorithms of leaf-level photosynthesis and transport within the canopy layers to estimate the gas exchange of each canopy layer (Lowman and Rinker, 2004). A variety of multilayer models differing

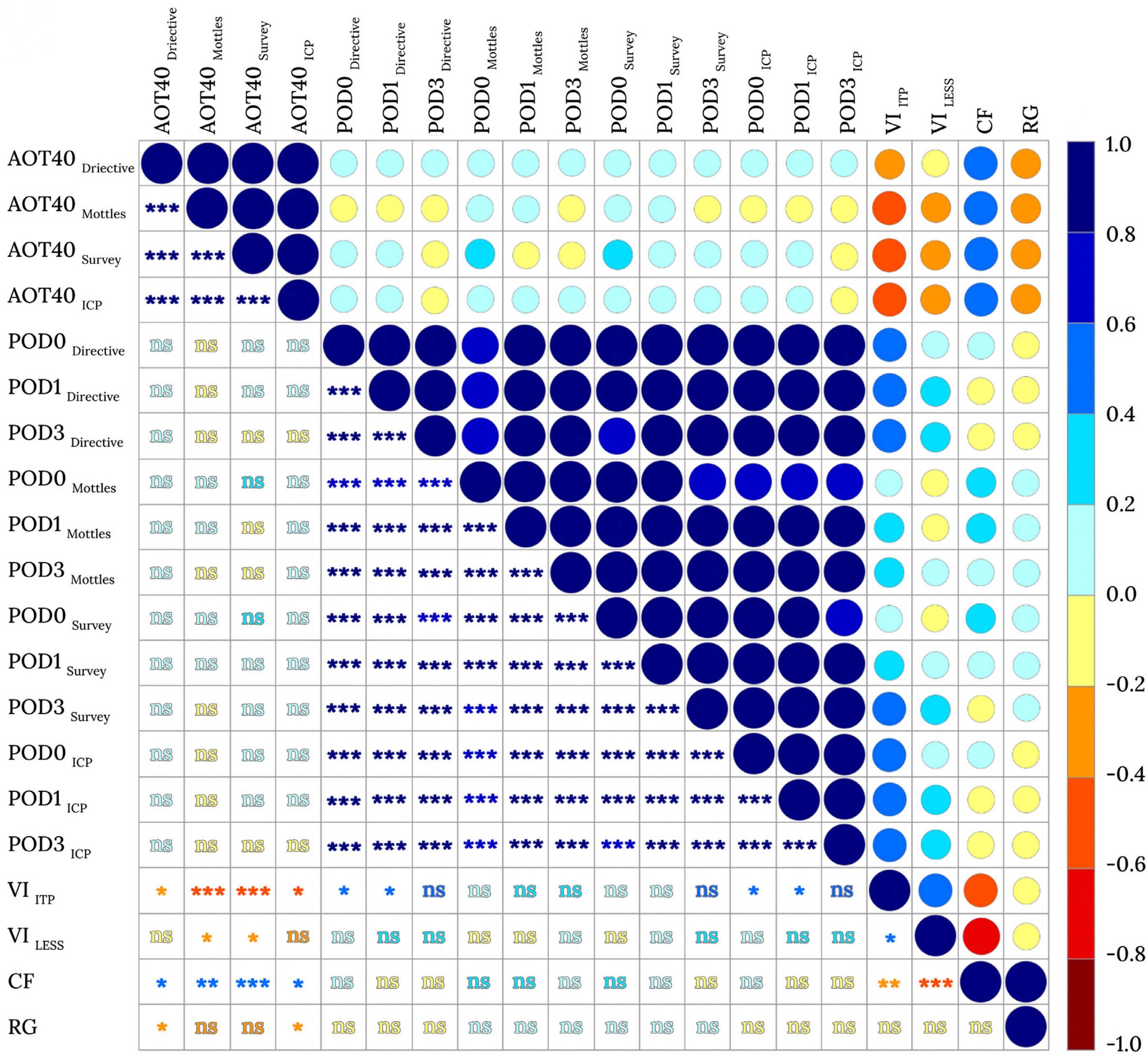

Fig. 1. Correlations of annual radial growth (RG), crown defoliation (CF), visible foliar ozone injury on the dominant species in the plot (VIITP) and along the LESS (VILESS), and ozone metrics (AOT40 and PODy). Spearman coefficients and level of significance p (*** p<0.001; ** p<0.01; * p<0.05; ns p> 0.05 not significant) for all 17 MOTTLES stations and the years over the time period 2017-2021. AOT40 is hourly ozone concentrations above 40 ppb, POD0, POD1 and POD3 and hourly stomatal ozone uptake above the threshold of 0, 1 and 3 mmol m^{-2} s^{-1}, respectively. The ozone metrics are accumulated from the actual start date of the growing season until the time of the survey, where. Ozone metrics are accumulated over four reference periods: Directive, i.e. April to September as recommended by Directive 2008/50/EC; Mottles, i.e. from start to end of the growing season; Survey, i.e. from start of the growing season to time of the survey; ICP, i.e. all-year round for Mediterranean evergreen species and April to September for the other species as recommended by CLRTAP (CLRTAP Mapping Critical Levels for Vegetation, 2017).

in spatial scale (i.e., local, regional and global model) and time resolution is available. For the purpose of O_3-risk assessment, an ideal model should adopt the most performing and mechanistic-oriented approach to simulate leaf species-specific stomatal conductance (g_s) and photosynthesis (A) resulting from interaction with O_3 exposure and microclimate (i.e., light, humidity and temperature) along the canopy profile (Lambers and Oliveira, 2019). To this end, Conte et al. (Conte et al., 2021) tested the effect of O_3 on a Holm oak forest in central Italy, using four flux-based O_3 impact response functions embedded in the multi-layer canopy model AIRTREE (Aggregated Interpretation of the Energy balance and water dynamics for Ecosystem services assessment, (Fares et al., 2019) developed to study forest ecosystem services such as carbon sequestration, O_3 and particle deposition. AIRTREE uses a coupled A-gs sub-model, based on the analytical solution of the Ball-Woodrow-Berry model proposed by Baldocchi, (Baldocchi, 1994) to simulate both canopy photosynthesis and stomatal flux of O_3. Using models which allow to simulate canopy cumulative fluxes offers an opportunity to test and evaluated them against Gross Primary Productivity (GPP) obtained from observations of Eddy Covariance fluxes of CO_2. This allows for instance to run models with and without accounting for possible O_3 damages in in-leaf periods under high O_3 exposure versus periods with low exposure to the pollutant. In order to improve the pre-

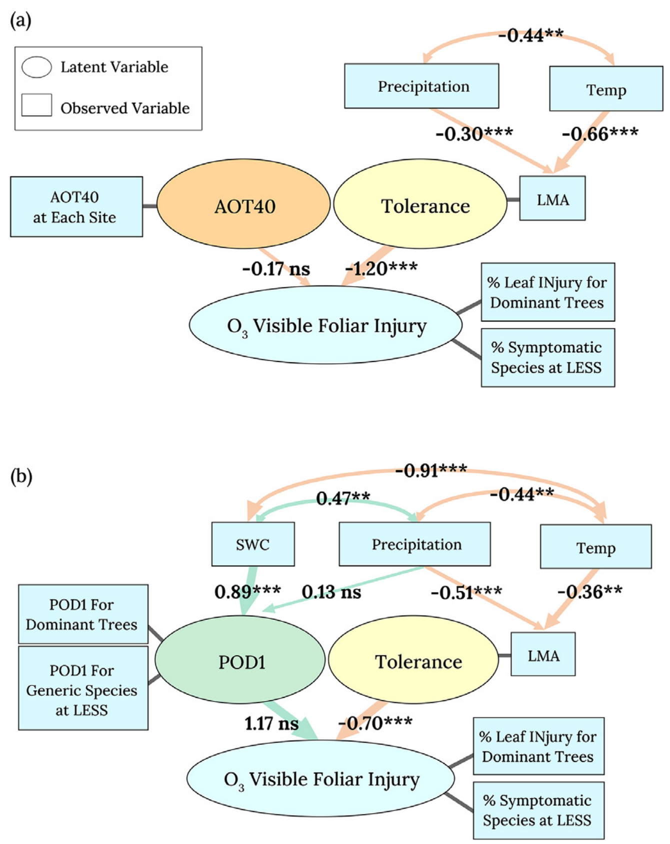

Fig. 2. Structural equation modeling (SEM) for O_3 visible foliar injury observed at forest monitoring sites in Italy. Two-way arrows denote correlations between two variables while one-way arrows denote causal relationships. Negative paths are shown by red lines while positive paths are shown by green lines. The values and size of the arrows denote the standardized SEM path coefficients indicating the magnitude of the strength of causality (z-test: *** p<0.001, ** p<0.01, * p<0.05, ns = not significant, N=37). The best model selection was conducted according to BIC (Bayesian information criterion). Four latent variables were used, i.e., Tolerance [characterized by LMA], AOT40 [characterized by AOT40 at each forest site], O_3 visible foliar injury [characterized by % Leaf injury for dominant trees and % Symptomatic species at LESS (Light-exposed sampling site)] and POD1 [characterized by POD1 for dominant trees and POD1 for generic species at LESS]. Following observed variables were used, SWC (soil water content), Temp (air temperature), Precipitation, LMA (Leaf Mass per Area), AOT40 at each forest site, POD1 for dominant trees (see details in (Sicard et al., 2020), POD1 for generic species at LESS (see details in (Sicard et al., 2021), % Leaf injury for dominant trees, and % Symptomatic species at LESS. The analysis by SEM was achieved by R software 4.1.2 (R Development Core Team, 2018).

dictive ability in simulating the ecophysiological impacts of O_3, Conte et al. (Conte et al., 2021) implemented the model with two multiplicative factors for A and gs calculated under controlled conditions of dose-response in a FACE experiment (Paoletti, 2007) using the same plant genotypes as proposed by Lombardozzi et al. (Lombardozzi et al., 2015). Thanks to the high temporal resolution of the model (30-minutes for all year), the authors evaluated if a clear phytotoxic threshold exists and if it changes during the year, testing six detoxifying thresholds ranging between 0 and 5 nmol O_3 m^{-2} s^{-1}. Interestingly, the authors concluded that POD1 is the best metrics to adopt for the studied ecosystem. However, the accumulation of stomatal fluxes above a threshold should change during the year to account for periods of high sensitivity of plants to O_3 exposure and therefore propose functions to describe a "dynamic-threshold" (Fig. 4). Changes in O_3 sensitivity during the day and in the course of the vegetative seasons have been indeed previously showed (Dizengremel et al., 2008; Luwe and Heber, 1995). The pioneer study by Conte et al. (Conte et al., 2021) took into account an evergreen oak

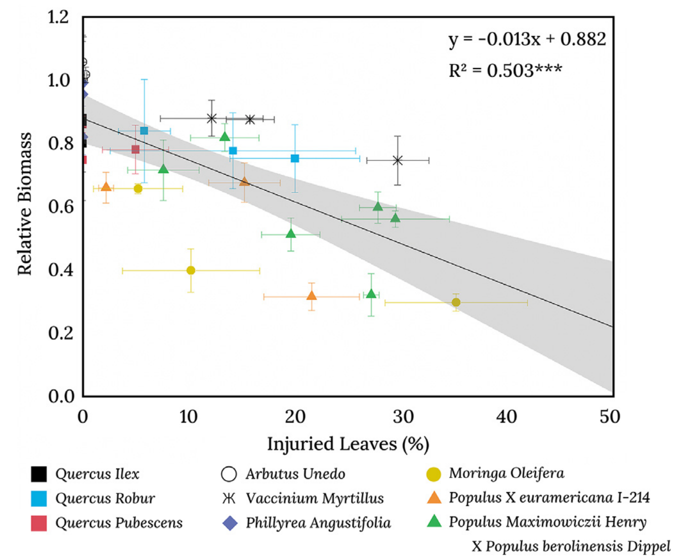

Fig. 3. Decrease of relative biomass with increasing visible foliar ozone injury in plants exposed to season-long ozone FACE experiments. The original results are published in (Moura et al., 2021; Hoshika et al., 2018; Zhang et al., 2018; Pellegrini et al., 2021; Hoshika et al., 2022)or unpublished (A. unedo, P. x euramericana). The total biomass is expressed as ratio of the control plants (exposed to ambient ozone exposure) versus the plants exposed to enriched ozone atmospheres (usually 1.5- and 2.0-times ambient ozone). The number of visibly injured leaves is expressed as a percentage of the total number of leaves. Bars show the standard errors. The linear regression line shows 95% confidence intervals in grey.

species, and may be replicated for other forest ecosystems to generate scalable functions and refine metrics based on stomatal O_3 fluxes. The advantage of this method is that it best uses data obtained from leaf-level measurements of gas exchange, manipulative experiments, meteorological data, and Eddy Covariance data, therefore a data assimilation effort which values previous experiments to better understand O_3 effects on vegetation carried out in the last decades. However, using multi-layer models for O_3-risk assessment requires species-specific information to build robust does-response relationship in order to obtain reliable results. This may in turn stimulate further research (i.e., new O_3 FACE experiments) to obtain more data also thanks to emerging technologies (i.e., high resolution sensors to capture photosynthesis and fluorescence) and implementation of monitoring networks using Eddy Covariance techniques with O_3 sensors. In this perspective, a worldwide network of Eddy Covariance monitoring systems (Rebmann et al., 2018, Kao et al., 2012) currently allows measuring of fluxes in continuous for years at high temporal resolution in a wide range of forest ecosystems and may represent a strong support in testing models.

3.3. Bridging experimental and monitoring research is needed for validation and parameterization of field results and models

To achieve a realistic risk assessment of O_3 impacts on forests, there is a growing need of successfully validating 'O_3-like' VFI which are surveyed during field monitoring. Although experimental exercises had been conducted by using controlled chambers especially for major dominant tree species such as Fagus sylvatica (Bussotti et al., 2007), there is still 'non-validated' VFI for several tree species. One of the reliable methods to study a realistic plant response to O_3 is a free-air controlled exposure (FACE) which has been developed as an advanced experimental approach (Montes et al., 2022, Neufeld and Perkins, 2021, Xu et al., 2021, Matyssek et al., 2007, Matyssek et al., 2013, Koike et al., 2013, Bassin et al., 2007). In fact, an O_3 FACE controls only O_3 concentrations

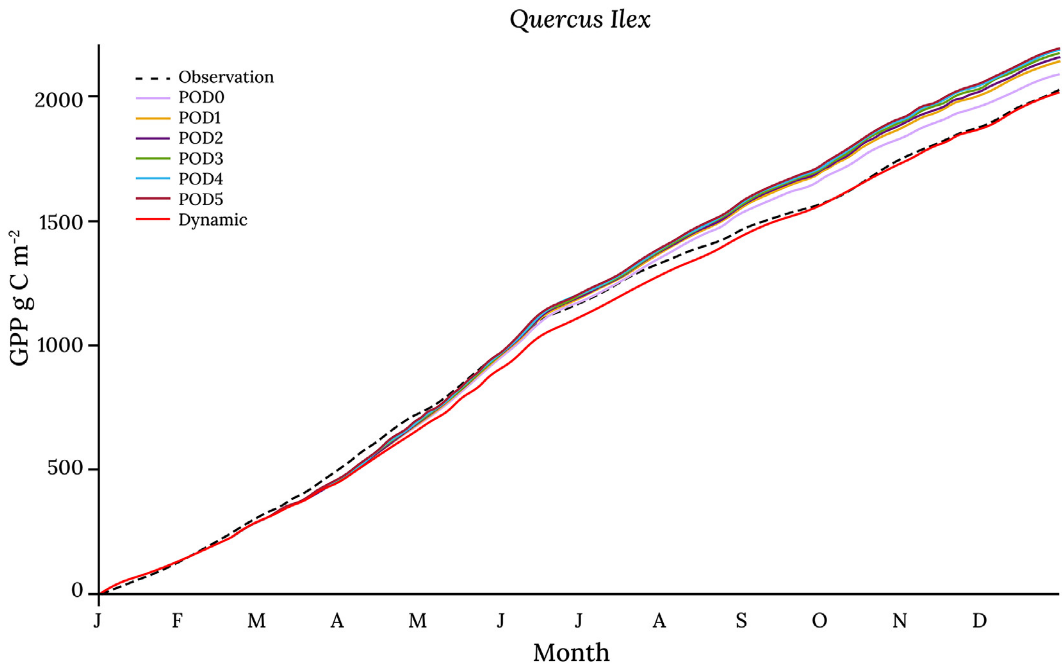

Fig. 4. Cumulative values of Gross Primary Productivity (GPP) calculated with the AIRTREE model for a Holm Oak (Quercus ilex) forest and different stomatal ozone uptake (POD) thresholds y and a dynamic threshold from January (J) to December (D). The dynamic threshold produced the best agreement between modelled and measured GPP. Data calculated based on results in Conte et al. (Conte et al., 2021).

while it minimizes the effect on the microenvironment such as solar radiation, wind speed and rainfall (Paoletti et al., 2017).

'O_3-like' VFI observed in the deciduous Alnus glutinosa, Sorbus aucuparia, Vaccinium myrtillus (Hoshika et al., 2020), and conifer species Pinus halepensis, P. pinaster and P. pinea (unpublished) during field monitoring in Italy were validated following one growing season exposure in an O_3 FACE facility (Fig. 3). One growing season of realistic O_3 exposure is needed, because VFI usually occurs towards the end of the growing season as the injury is cumulated over time (Hoshika et al., 2020). Three levels of O_3 treatments were applied: AA, ambient O_3 concentration; 1.5×AA, 1.5 times ambient O_3 concentration; and 2.0×AA, twice ambient O_3 concentration. These enhanced O_3 treatments properly reproduced the VFI caused by O_3. For example, P. halepensis needles exhibited a chlorotic mottling, A. glutinosa, S. aucuparia and V. myrtillus showed the red or dark brown stippling among the mid veins on the upper leaf surface which were actually observed at the MOTTLES monitoring sites. The newly validated VFIs are available at the MOTTLES O_3 injury website (https://mottles-project.wixsite.com/life/atlas-ozone-injury) which contains a photographic collection of VFIs. These kinds of web data base (see also "Ozone injury in European Forest Species" http://www.ozoneinjury.org/) are helpful tools for supporting the real-world quantification of O_3 injury and thus the verification of CL exceedances.

In addition to the validation of VFI, O_3 FACE may also be utilized to develop the species-specific DO3SE model parameters for estimating PODy for target species. In fact, the DO3SE parameters are currently available only for some major European tree species in the ICP Vegetation manual (e.g., F. sylvatica, Picea abies (CLRTAP Mapping Critical Levels for Vegetation, 2017), although 23 symptomatic species were recorded in three countries of the MOTTLES network (Paoletti et al., 2019). Hoshika et al. (Hoshika et al., 2020)conducted measurement campaigns for the new parametrization of the model in two tree species that were the dominant species at MOTTLES sites (alder: Alnus glutinosa in France, and phyllirea: Phyllirea latifolia in Italy) and two symptomatic species found over the LESS (mountain ash: S. aucuparia, bilberry: V. myrtillus). The new parameterization of the DO3SE model can consider various species-specific ecological and physiological characteristics to estimate the stomatal dose of O_3. For example, the maximum stomatal conductance (gmax), which is the most important parameter for the estimation of PODy, was relatively high in the water-demanding alder (300 mmol O_3 m^{-2} PLA s^{-1}) as compared to the other three species (phyllirea:150 mmol O_3 m^{-2} PLA s^{-1}, mountain ash: 240 mmol O_3 m^{-2} PLA $s^{-1}$and bilberry: 140 mmol O_3 m^{-2} PLA s^{-1}). Such a high gmax was also reported in other O_3 sensitive species such as Oxford poplar clone (approximately 340 mmol O_3 m^{-2} PLA s^{-1}) (Hoshika et al., 2018). In fact, a relatively high VFI (approximately 5%) was recorded in alder trees at the French site because a high gmax promotes stomatal O_3 flux, thus causing O_3 injury.

Manipulative experiments such as FACE provide insights into the physiological mechanisms of tree responses to O_3 at a realistic scale while forest monitoring delivers a real-world assessment of VFI. The combination of these two approaches makes it possible to realize biologically meaningful VFI-based CLs for the protection of forests from O_3.

3.4. There is a need to reconcile present critical levels and dose-response functions to estimate O_3-induced biomass losses

To encourage a reanalysis of biomass-based CLs and the use of VFI-based CLs, we show here new results about a clear discrepancy when comparing CL exceedances calculated from WRF-Chem results and biomass losses estimated on the basis of the presently available dose-response functions for different forest functional types. Chemical Transport Models are helpful to simulate O_3 concentrations and meteorological variables with hourly time resolution and different spatial resolutions (de Marco et al., 2022). As demonstrated at different scales, from local to regional or hemispheric level, extent and distribution of potential O_3 risk areas for vegetation are very different when estimated by the exposure- or flux-based approaches. Indeed, De Marco et al. (de Marco et al., 2015) compared the local-scale spatial and temporal distribution of AOT40 and PODy for a conifer (Pinus halepensis) and a deciduous broadleaf tree species (Fagus sylvatica) over a South-European domain, and found that the exposure-based AOT40 suggested a larger spatial distribution of O_3 risk relative to the flux-based PODy. At the regional

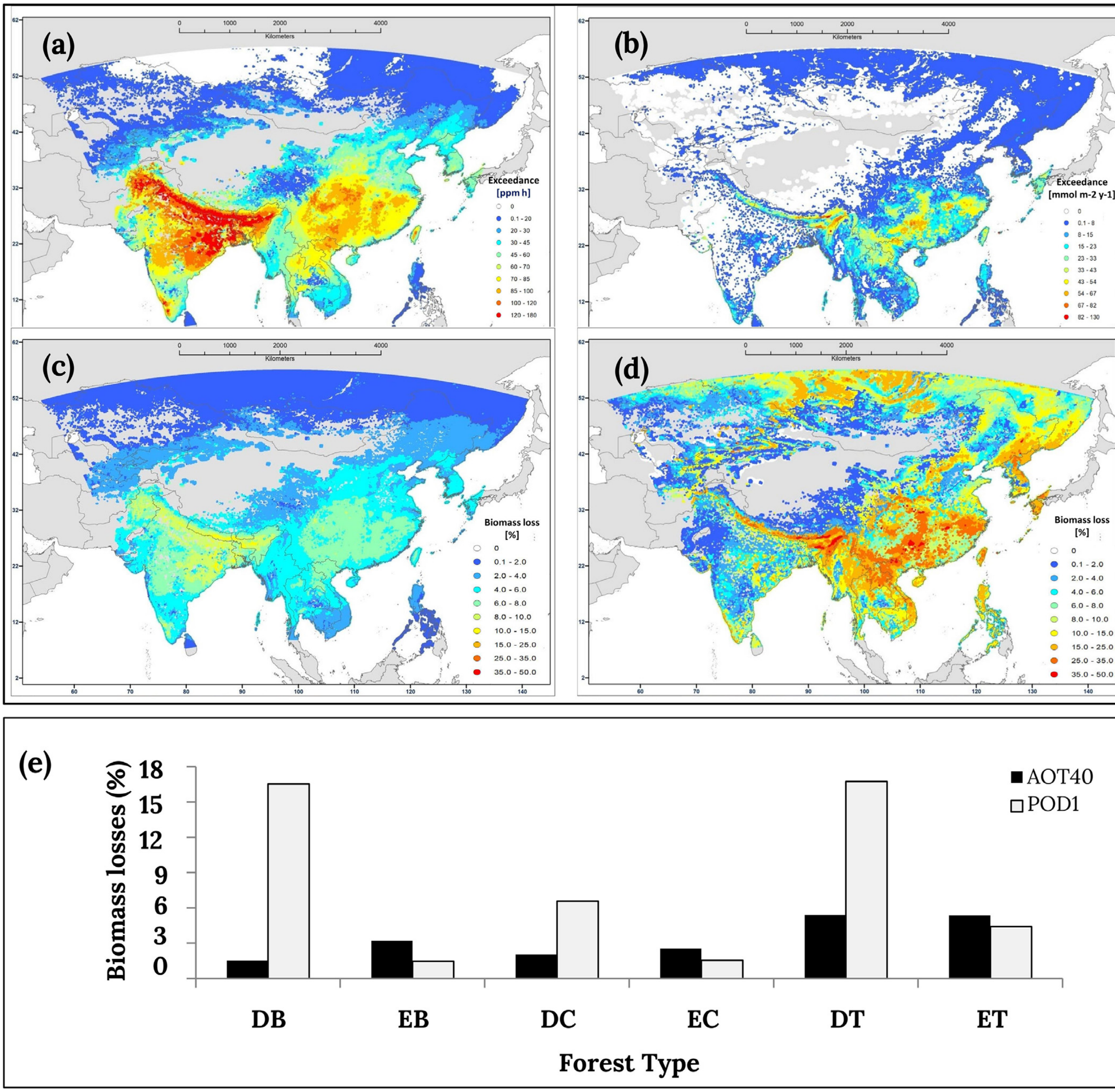

Fig. 5. Exceedances of the critical levels (a, b) and biomass losses (c, d, e) for AOT40 (a, c) and POD1 (b,d) in the year 2015. Exceedances were calculated from the WRF-Chem results in (de Marco et al., 2020) and biomass losses were estimated on the basis of the dose-response functions in (CLRTAP Mapping Critical Levels for Vegetation, 2017)for the six forest categories in De Marco et al. (de Marco et al., 2020) i.e. boreal deciduous (BD), boreal evergreen (BE), continental deciduous (CD), continental evergreen (CE), (sub)tropical deciduous (TD), and (sub)tropical evergreen (TE) species.

scale, modelled AOT40 and POD1 values showed large differences in their spatial distribution over Europe (Anav et al., 2016), moving the hot-spot region from the Mediterranean area (AOT40) to the Atlantic region (POD1), due to the large limitation to stomatal uptake in the Mediterranean region. This response is largely due to SWC, that is a typical limiting factor for stomatal uptake under dry and semi-dry climatic conditions. In Asia, AOT40 was found to underestimate the O_3 risk to deciduous forests and overestimate it to evergreen forests when compared to POD1 (Sicard, 2021, de Marco et al., 2020). Over this Asian domain, the AOT40 CL of 5 ppm h (CLRTAP 2004) was exceeded over 53%, 93%, 74%, 86%, 98% and 97% of the areas covered by boreal deciduous, boreal evergreen, continental deciduous, continental evergreen, sub-tropical deciduous and sub-tropical evergreen forest types, respectively. The most limiting factors in O_3 uptake were light availability (65%), SWC (29%) and air temperature (6%), making this region at high O_3 risk for deciduous species and at medium O_3 risk for evergreen species. The findings obtained at local and regional scale are confirmed by recent observations at northern hemispheric level (Anav et al., 2022). Modelling O_3 concentration and fluxes over such large area showed that AOT40 substantially overestimates the extension of potential vulnerable regions, predicting that 61% of the forested area in the northern hemisphere are at risk of O_3 pollution. Conversely, POD1 identified lower extension of vulnerability regions (40%).

A major open question, however, is whether the above-mentioned discrepancies in the spatial distributions of AOT40 and POD1 translate into similar discrepancies in the forest biomass losses (Juráň et al., 2018)

. Complex land surface model such as ORCHIDEE can estimate gross primary production (GPP) losses due to O_3 fluxes. Anav et al. (Anav et al., 2022) found that present O_3 uptake decreases GPP in 37.7% of the forested area of the northern hemisphere with a mean loss of 2.4% per year. Here we applied the methodology by De Marco et al. (de Marco et al., 2020) to estimate the spatial distribution of annual AOT40- and POD1-based exceedance values over the same Asian domain, and innovatively used the dose-response functions of Table 1S for estimating forest biomass losses for the different forest types identified into each grid of the selected domain (de Marco et al., 2020).

Exceedances were calculated based on the CLs for forest protection, set to 5 ppm h in the case of AOT40 and to different values for different forest types in the case of POD1, namely 5.2 mmol m^{-2} for boreal and continental deciduous forests, 9.2 mmol m^{-2} for boreal and continental evergreen forests, 14.0 mmol m^{-2} for sub-tropical deciduous species and 47.3 mmol m^{-2} for sub-tropical evergreen forests (CLRTAP Mapping Critical Levels for Vegetation, Chapter III 2017). A larger exceedance area was estimated for AOT40 (88%, Fig. 5a) than for POD1 exceedances (57%, Fig. 5b). For the biomass losses, the situation was completely different, with lower losses for AOT40 (3±1%, Fig. 5c) than for POD1 (7±5%, Fig. 5d). This large difference was mainly due to deciduous species, while the biomass losses calculated with both methodologies for evergreen species were very similar (Fig. 5e). These results can be explained based on the recognized different sensitivity of deciduous and evergreen (sub)tropical species to O_3 impacts (Li et al., 2017). However, the dose-response functions still need to be validated by ozone FACE experimental data, to overcome possible artifacts due to artificial conditions in closed chambers.

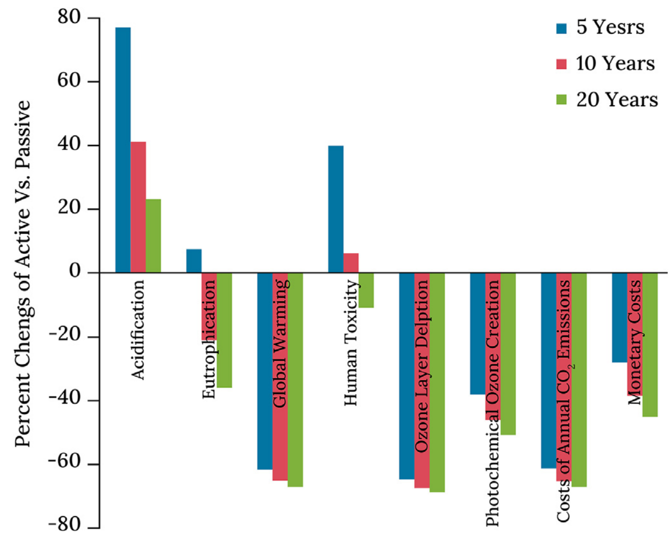

Fig. 6. Percent changes when using active monitors rather than passive sensors for monitoring atmospheric ozone concentrations at remote forest sites (400 km distance from the research center). The data for the potential of acidification, eutrophication, O_3 depletion, global warming, human toxicity and photochemical O_3 creation as well as annual CO_2 emissions (3% discount rate) and monetary costs, are re-elaborated from Carrari et al. (Carrari et al., 2021) by averaging results of evergreen and deciduous forest sites in Italy.

3.5. *Active ozone monitoring is a cost-effective approach for estimating stomatal ozone flux and its economic effects on forests*

Based on MOTTLES monitoring data, evidence was provided that the environmental, economic, and social costs of active O_3 monitoring are lower than those of passive sensors (Carrari et al., 2021), suggesting that passive monitoring is not environmentally sustainable in terms of O_3 depletion, global warming, and photochemical O_3 creation potential, especially over long time periods (Fig. 6). In Italy, i.e., a country with relatively high labor costs, passive monitoring was more expensive, especially in forests with long growing season (evergreen) and thus more travels for the periodic gathering of passive samplers.

PODy monitoring may also help a quantitative assessment of the impacts on forest ecosystem services. Among the many ecosystem services provided by forests, only wood production losses have been estimated so far (Feng et al., 2019; Felzer et al., 2005; Karlsson et al., 2005), because experimental dose–response relationships are available for POD1 (Gao et al., 2017; Hoshika et al., 2018; Büker et al., 2015; Hoshika et al., 2012). Sacchelli et al. (Sacchelli et al., 2021) developed an approach for integrating fine-scale O_3 risk modelling and economic estimates, by using MOTTLES data and the Italian forests as a case study. Results suggested a significant impact of O_3 expressed as POD1 with a loss of capital value of about 10%. There was also a 1.1% reduction in the profitable forest areas, i.e., with a positive Forest Expectation Value (FEV). This loss of economic profitability may make active forest management no longer meaningful in O_3-polluted forests, and potentially result in indirect negative effects on other ecosystem services.

4. Conclusions

Monitoring the negative impacts of O_3 pollution upon forest ecosystems requires cost-effective and risk-based approaches. Stomatal O_3 flux accumulated over the growing season is demonstrated to be an optimal tool to assess O_3 injury to real-world vegetation and establishing CLs. So far, CLs have used biomass losses as forest-health indicator, and the biomass losses were estimated in experimental facilities. Ozone-induced biomass losses of adult forests, in contrast, are difficult to assess. In fact, a clear discrepancy emerges when comparing CL exceedances calculated from WRF-Chem results and biomass losses estimated on the basis of the presently available dose-response functions for different forest types. The traditional focus on biomass-based CLs may be due to the economic value of wood, but we should now consider that forests provide many other valuable ecosystem services in addition to wood production. We stress a need to reconcile present CLs and dose-response functions by using the realistic ozone FACE facilities to estimate O_3-induced biomass losses and to change the paradigm of biomass loss based CL and use VFI recorded annually in large-scale forest surveys coupled with active O_3 monitoring. For a proper use of VFI in setting epidemiology-based CLs for forest protection, active O_3 monitoring as well as validation and parameterization of field results and models by the ozone FACE facilities are needed. Interestingly, VFI is well correlated to O_3-induced biomass loss at O_3 FACE experiments, and POD1 and plant O_3 tolerance affect VFI under field conditions.

POD1 shows a much stronger causality than AOT40 in affecting VFI. Accurate calculation of PODy at field sites is now possible, thanks to recent technological advances that support low-cost and reliable monitoring of PODy at forest sites and thus ensure long-term sustainability of such laborious monitoring. Active O_3 monitoring is a cost-effective approach for estimating PODy, quantifying the economic impacts of O_3 on forest health and sustainability, and allowing respect the developments of environmental legislation. These results support long-term sustainability of PODy monitoring at forest sites and warrant further studies on the responses of VFI to POD1 under field conditions, species-specific CLs, multi-layer canopy models and estimations of POD1-induced reduction in wood production and other forest ecosystem services.

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Elena Paoletti: Conceptualization, Supervision, Funding acquisition, Writing – original draft. **Pierre Sicard:** Funding acquisition, Data curation, Formal analysis, Methodology, Writing – original draft. **Yasutomo Hoshika:** Data curation, Formal analysis, Writing – original draft. **Silvano Fares:** Funding acquisition, Investigation. **Ovidiu Badea:** Supervision, Funding acquisition, Writing – review & editing. **Diana Pitar:** Data curation, Formal analysis. **Ionel Popa:** Data curation, Formal analysis. **Alessandro Anav:** Validation. **Barbara Baesso Moura:** Formal analysis, Writing – review & editing. **Alessandra De Marco:** Data curation, Formal analysis, Writing – original draft.

Acknowledgments

The projects MOTTLES (LIFE15 ENV/IT/000183), coordinated by IRET-CNR, as well as NEC Italia and MODERN (NEC) (LIFE20 GIE/IT/000091), coordinated by CUFAA, are greatly acknowledged. A special acknowledgment is to the MOTTLES project manager Elisa Carrari and FTP responsible Francesco Sabatini, as well Jacopo Manzini for support in updating the data base and Cristina Mascalchi for logistic and administrative support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.horiz.2022.100018.

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