*Title Page

The effects of ozone on oaks: a global meta-analysis

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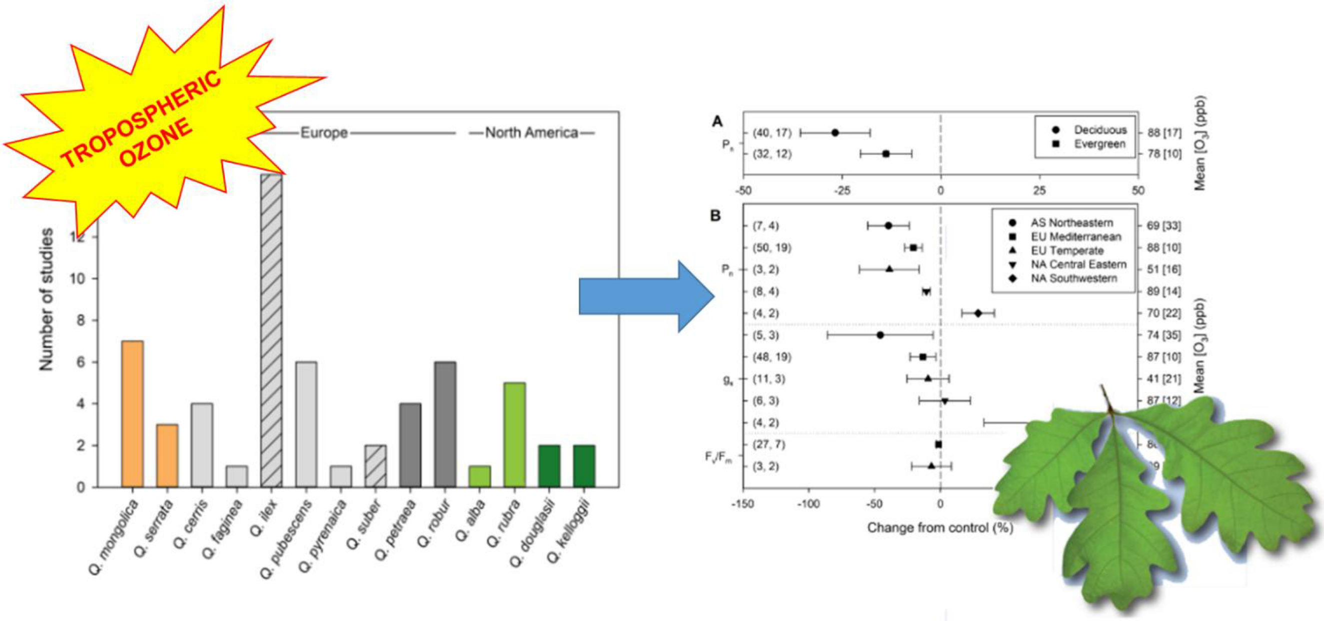
***Graphical Abstract**



*Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

Highlights

1. Tropospheric ozone (O_3) levels in air are elevated, and are predicted to increase
2. This meta-analysis reports the O_3 effects on 51 parameters of 14 oak species
3. Oaks are tolerant to O_3 in terms of biomass, but physiological impairment occurred
4. Deciduous species and oaks native to Eurasia are less tolerant to O_3
5. Negative effects induced by drought seemed not to be exacerbated by O_3

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Abstract

Tropospheric ozone (O_3) levels in air are still elevated in many regions of the world including Northern Hemisphere forests, and are predicted to increase further due to both anthropogenic activities and climate change. This meta-analysis shows overwhelming evidence of the O_3 effects on 51 growth, anatomical, biomass, physiological and biochemical parameters of 14 deciduous or evergreen oak species distributed all around the Northern Hemisphere. Although no large impacts were observed on biomass, suggesting an O_3 tolerance by oaks, some impairments were found at physiological level that might negatively affect both carbon sequestration and water vapour transfer to the atmosphere. This outcome suggests the need to fully incorporate this phenomenon into future projections of how atmospheric change and forest biomes will interact in effecting future climatic change. Among the antioxidants used by oaks to respond to O_3, phenols seems to have a crucial role. Deciduous species resulted more affected by O_3 than evergreen ones, as well as oaks native to Eurasia, in comparison with those from North-America. Experiments performed in less controlled environments showed more O_3 deleterious effects, especially under higher AOT40 levels, but negative impacts were also reported for acute O_3 exposures. Most of the reviewed studies with additional treatments to O_3 exposure investigated the interaction(s) between O_3 and drought, but the negative effects induced by water deprivation seemed not to be exacerbated by the pollutant. However, more combined experiments on the impact of O_3 and co-occurring stressors on woody

species are necessary. Another major issue highlighted by the present study is the lack of experiments on adult trees. To better understand O_3 impacts, and to reinforce the strength of O_3 impact predictions, O_3 controlled experiments on young individuals should be combined with long-term experiments on mature trees grown in open-air conditions.

Keywords:

Air pollution, C sequestration, deciduous/evergreen forests, global change, photosynthesis, *Quercus*.

1. Introduction

Tropospheric ozone (O_3) is a widespread secondary air pollutant (Monks et al., 2015). Although several efforts have been made to reduce the emission of its precursors, mainly nitrogen oxides and volatile organic compounds, O_3 levels in air are still elevated in many regions of the world, and are predicted to increase further due to both anthropogenic activities and climate change (Lefhon et al., 2018). Background O_3 concentration is predicted to rise up to 42-84 ppb in 2100, depending on the time-space variability, with occasionally peaks exceeding 200 ppb (1 ppb = 1.96 µg m^{-3}, at 25 °C and 101.325 kPa; IPCC, 2014; Yang et al., 2018). This raise is especially expected in hot-spot areas such as East Asia and the Mediterranean basin (Verstraeten et al., 2015; Ochoa-Hueso et al., 2017), although opposite trends are also foreseen in other regions (Watson et al., 2016; Sicard et al., 2017). At elevated concentrations, O_3 can severely influence global environmental change and negatively affect many biological activities in humans, animals, and plants (Bhuiyan et al., 2018; Nuvolone et al., 2018; Ainsworth, 2017).

The detrimental impact of O_3 on forests has been well documented in North America (e.g., Chappelka and Samuelson, 1998; Fenn et al., 2020), Europe (e.g., Paoletti, 2006; De Marco et al., 2017) and Asia (e.g., Koike et al., 2013; Li et al., 2017) in terms of visible injury, reductions in biomass, changes in biomass partitioning, or a higher susceptibility to pathogen challenge (Calatayud et al., 2011), although perplexities and lack of evidence of these negative effects on forest ecosystems have been also reported (Cailleret et al., 2018). As a strong oxidant, O_3 has a negative impact on many cellular and molecular processes. Among others, O_3 commonly induces an alteration of photosynthetic performance with reduction in stomatal conductance, cell dehydration, and high production of reactive oxygen species (ROS), leading to leaf chlorosis and early senescence (Ainsworth, 2017). However, plants are able to cope with the O_3 induced oxidative pressure by regulating a complex network of enzymatic and non-enzymatic antioxidant compounds (Gill and Tuteja, 2010; Pellegrini et al., 2018; Marchica et al, 2019). Although the large number of published studies focused on O_3-plant interaction, establishing the vulnerability of vegetation to rising O_3 concentrations is complicated by marked differences in findings between individual studies. Meta-analytic techniques may provide an objective means to quantitatively summarize treatment responses (Morgan et al., 2003).

First meta-analyses on the effects of O_3 on woody species (Grantz et al., 2006; Wittig et al., 2007; Wittig et al., 2009), as well as on crops (Morgan et al., 2003; Grantz et al., 2006; Feng et al., 2008; Feng and Kobayashi, 2009), date back to more than ten years ago. Grantz et al. (2006) performed a meta-analysis on O_3 impacts on root/shoot allocation and growth of a number of herbaceous plants, but also including five tree species. Pioneering meta-analytic papers by Wittig et al. (2007, 2009) synthesized the knowledge about O_3 impacts on tree biomass, growth, physiology and biochemistry by using studies performed in North America and Europe, on angiosperms and gymnosperms, from temperate and boreal climates. Results indicated that elevated O_3 concentrations reduced leaf photosynthetic CO_2 uptake, stomatal conductance (Wittig et al., 2007), and total biomass (Wittig et al., 2009). Recently, Li et al. (2017) performed a similar meta-analysis on the responses of Chinese woody plants to background O_3 concentrations, highlighting that temperate species from China were more sensitive to O_3 than those from Europe and North America in terms of photosynthesis and transpiration; and that subtropical species were significantly less sensitive to O_3 than temperate ones; whereas deciduous broadleaf species were significantly more sensitive to O_3 than evergreen broadleaf and needle-leaf species. Even more recently, Feng et al. (2019) focused on the effects of current and future elevated O_3 concentrations on poplar, showing that current O_3 concentration significantly reduced CO_2 assimilation rate and total biomass, and that an increase in future O_3 concentrations would further enhance the reduction in total biomass, plant height and leaf area. Despite the interesting outcomes provided by these meta-analyses, several points remain to be addressed and updated, with studies regarding the effects of O_3 on specific important genera, such as *Quercus*, not summarized in detail so far.

Oaks (more than 500 species of trees or shrubs in the genus *Quercus*, Fagaceae) are among the most important woody angiosperms in the Northern Hemisphere in terms of species diversity, ecological dominance, and economic values (Nixon, 2006). Oaks are dominant members of a wide variety of habitats, including temperate deciduous forest, temperate and subtropical evergreen forest, subtropical woodland, oak-pine forest, tropical premontane and montane forest, and a variety of Mediterranean climate vegetation, such as oak woodland and evergreen oak forest (Denk et al., 2017). Although many oak species are exceptionally large, dominant overstory trees, perhaps an almost equal number of species are shrubs or small trees. The economic importance of oaks in the Northern Hemisphere is widely known. Various species are sources of high-quality lumber, and oak firewood is the preferred in many areas, particularly as a cooking/heating fuel (Nixon, 2006). Since the 70's (e.g., Treshow and Stewart, 1973), a number of studies have been carried out to investigate the effects of O_3 on the growth, biomass, physiology and biochemistry of oaks native to several regions worldwide (see Table S1). However, a general understanding of oaks susceptibility to O_3 threat is still lacking.

The objective of this meta-analytic paper is to summarize and synthesize the outcomes of numerous studies on growth, anatomy, biometrics, physiology and biochemistry of oaks in response to O_3

pressure. The following questions are specifically addressed: (i) What are the overall O_3 effects on oaks? (ii) Do the O_3 effects differ for plant type, species native area, plant age, exposure comparison type [e.g., plants exposed to ambient air (i.e., current O_3 concentrations) or elevated O_3 concentrations (i.e., projected for the future) relative to plants kept under charcoal-filtered air], exposure method, O_3 severity and presence of additional treatments?

2. Materials and methods

2.1. Database

A database of variations of growth, biomass, physiology, and biochemistry traits of oaks under O_3 was created by examining the published peer-reviewed literature, searched in the Web of Science (Thompson-ISI, Philadelphia, PA, USA, http://apps.webofknowledge.com/) and Scopus (Elsevier, Amsterdam, Netherlands, http://www.scopus.com/) databases, using "ozone", "oak" and "*Quercus*" as keywords. Database searches were performed on May 2020 and were made back to 1971. The reference lists of any article identified by this literature search were cross-checked in order to include any other relevant reference, finally identifying 140 research papers focused on any interaction of oaks with O_3. Articles and their data were excluded if (i) the focus was not on the effects of O_3 on growth, biomass, physiology and biochemistry of oaks (e.g., O_3 fluxes, determination of critical levels for O_3 risk assessment, atmospheric O_3 biomonitoring, O_3 removal by forests, transcriptome analysis, O_3 sanitation), (ii) there was no replication or the standard deviation could not be determined (only 3% of the original selected papers), (iii) there was no experimental control (i.e., plants kept under charcoal-filtered or ambient air), (iv) O_3 concentration and/or AOT40 were not specified or calculable (if AOT40 was not reported in the original paper, it was calculated by O_3 mean concentration, hours of fumigation per day and days of exposure). After this article removing, 40 research papers including 14 oak species were included in the present meta-analysis (Table S1). The articles were examined for any growth, biomass, anatomical, physiological and biochemical parameters. For each trait observation, the mean values under control or elevated O_3 conditions ($\overline{X}_C$ and $\overline{X}_{O3}$, respectively), as well as their standard deviations and number of replications, were directly obtained from table or text, if reported; otherwise, they were extrapolated from graphs using the GetData Graph Digitizer (v. 2.26; http://getdata-graph-digitizer.com/). Values collected on plants under charcoal-filtered air, if present, were used as controls, otherwise effects induced by increased O_3 concentrations were determined in comparison with plants exposed to ambient air. All these values were associated in the database with the categorical information (see the 'sources of variation' section; Table S1), including the concentration and duration of the O_3 exposure. The World Flora Online (http://www.worldfloraonline.org/) and the work by Denk et al. (2017) were used to classify the plant type (deciduous and evergreen) and native areas of oak species.

Since methods for meta-analyses require that single observations are statistically independent, trait values were recognized independent if they were collected on different species or subspecies within a species, under different O_3 concentrations, additional treatments, or if the measurements were performed at different years in the same experiment, following previous meta-analyses (Morgan et al., 2003; Wittig et al., 2007; Feng et al., 2019). Parameter values were collected at the end of exposures. If gas exchange measurements were made over the diurnal course, only values for light-saturating conditions were included in the dataset. Nine of the selected articles included more than one experimental group (i.e., plants exposed to different O_3 concentrations higher than controls); thus, in order to avoid/limit non-independence issue due to shared control among experimental groups, and at the same time to remove information as little as possible, we proceeded as follows: if AOT40s among experimental groups were within consecutive classes (see below) and/or less than six observations were collected, we removed the experimental group exposed to the lower AOT40 value (suggested option); otherwise we split the shared control into two groups with halved sample size (only applied to two articles since non-suggested option; Gurevitch and Hedges, 1999; Noble et al., 2017; Higgins et al., 2019).

2.2. Sources of variation

The response of oaks to elevated O_3 concentrations was investigated for the following seven categories: (i) plant type (evergreen and deciduous), (ii) native area of species (Asia-Northeastern, Europe-Mediterranean, Europe-Temperate, North America-Central Eastern and North America-Southwestern), (iii) plant age (young and adult), (iv) exposure method (Closed Exposure Chambers, CEC; solardomes; Open Top Chambers, OTC, branch chambers; and Free Air Controlled Exposures, FACE), (v) exposure type comparison (charcoal-filtered air *vs* elevated O_3 concentrations, CF *vs* E; charcoal-filtered air *vs* ambient air, CF *vs* AMB; and ambient air *vs* elevated O_3 concentrations, AMB *vs* E), (vi) Accumulated Ozone exposure over a Threshold of 40 ppb (AOT40) over the entire experiment (five classes were determined: 0–5, >5–15, >15–25, >25–35, and >35 ppm h), and (vii) additional treatments (no additional treatments, drought, high CO_2 concentration, nitrogen addition, and salinity). Since 98% of the studies were carried out on potted plants, the rooting environment was not categorized.

2.3. Meta-analyses

To perform the meta-analysis, we used the software OpenMee (Brown University, Providence, RI, USA; Wallace et al., 2017), and the natural log of the response ratio, calculated as $r = \ln(\overline{X}_C/\overline{X}_{O3})$, was used as the metric for estimating the O_3 effect, according to Rosenberg et al. (2000). According to previous meta-analyses (Feng et al., 2008, 2019; Wittig et al., 2007, 2009; Li et al. 2017), effect sizes are reported as the unlogged *r* converted to the mean percentage change from the control as $(r - 1) \times 100$. Negative percentage changes indicate a decrease in the variable in response to elevated O_3 treatment, while positive values indicate an increase. Based on the assumption of random variation in effect sizes between studies, we used a weighted mixed-model analysis, where each individual response was weighted by the reciprocal of the mixed-model variance (Gurevitch and Hedges, 1999; Hedges, Gurevitch, and Curtis, 1999; Wittig et al., 2007). Effect size estimates were considered significant when the 95% CI did not overlap zero (Feng et al., 2008). Parameters were included in this analysis if there was a minimum of 9 observations; otherwise, parameters were only included if they originated from two or more independent papers, in order to make the results more robust, according to previous meta-analysis experiences (e.g., Feng et al. 2008, 2019; Wittig et al. 2007, 2009)

For each category listed in the sources of variation, Q_B was assessed, and if Q_B was significant ($P < 0.05$), data were subdivided based on the levels of those categorical variables (i.e. meta-regression). Means were considered significantly different among them when there was no overlapping of the 95% CI (Feng et al., 2019). Levels of each category were included in this analysis if there was a minimum of 9 observations. If less than 9 observations were available, results were only discussed if they originated form two or more independent papers, in order to make the results more robust, according to previous meta-regressions (e.g., Feng et al., 2008; Wittig et al., 2007, 2009).

3. Results

3.1. Overview on the database of the O_3 effects on global oak species

The 40 studies selected for the present meta-analysis (Appendix A) were performed around all continents of the Northern Hemisphere, with most of the studies carried out in Europe, especially within the Mediterranean area (Figure 1). First experiments on oak-O_3 interaction were performed in North America, followed by investigations in Europe, and more recently in Asia (Table S1). Fourteen oak species were investigated in these studies: *Q. mongolica* and *Q. serrata* native to Asia-Northeastern, *Q. cerris*, *Q. faginea*, *Q. ilex*, *Q. pubescens*, *Q. pyrenaica* and *Q. suber* native to Europe-Mediterranean, *Q. petraea* and *Q. robur* native to Europe-Temperate, *Q. alba* and *Q. rubra* native to North America-Central Eastern and *Q. douglasii* and *Q. kelloggi* native to North America-Southwestern. Among these species, only the Mediterranean *Q. ilex* and *Q. suber* are evergreen (Figure 2, Table S1). Oak species native to Europe-Mediterranean were the most studied (especially *Q. ilex*), followed by those native to Europe-Temperate and Asia-Northeastern, and then by those native to North America-Central Eastern and -Southwestern (Figure 2, Table S1). Only five studies (12.5%) investigated adult trees (Table S1). Most of the reports (62.5%) investigated the effects of (quite) elevated O_3 concentrations (mean: 76.5 ppb; with a minimum of 35 ppb and a maximum of 250 ppb for acute exposures) relative to charcoal-filtered air (mean: 12.5 ppb; range: 0-25 ppb), whereas 27.5% of the studies examined the impact of elevated O_3 concentrations relative to ambient air (mean: 32.5 ppb; range 28-42 ppb), only one study focused on the effects of ambient air relative to charcoal-filtered air, and three studies investigated both elevated O_3 and ambient air in comparison with charcoal-filtered air (Table S1). The total duration of the experiments ranged from one day to around two years. Most of the studies evaluated the highest class of AOT40 (i.e., >35 ppm h), alone (40%) or including other AOT40s (12.5%); whereas 30% of the studies investigated the lowest AOT40 classes (i.e. 0-5 or 5-15 ppm h). Most of the studies were performed by OTC (37.5%) or CEC (35%), followed by those carried out by FACE (20%); only two and one studies used solardomes or branch chambers, respectively (Table S1). Half of the works did not include an additional treatment to O_3 exposure, whereas 20% and 15% of the papers involved drought and high CO_2, respectively; three studies incorporated both drought and high CO_2, two of them included nitrogen addition, and only one included salinity (Table S1).

3.2. Overall O_3 effects

Overall O_3 effects on growth, anatomical, biomass, physiological and biochemical parameters of oaks included in the present meta-analysis (n = 51) are shown in Figure 3 and Table S2. Across all studies, leaf area and stomatal density increased by 33 and 6% due to O_3 (under 62 and 87 ppb, respectively; mean O_3 concentrations are reported, as well as hereinafter), in comparison with control. Conversely, O_3 (39 ppb) decreased aboveground biomass by 11%. In addition, net photosynthesis (P_n), transpiration (E), stomatal conductance (g_s) and maximum rate of Rubisco carboxylation (V_{cmax}) were negatively affected by O_3 (-21, -47, -13 and -27%, under 84, 116, 79 and 66 ppb, respectively), as well as maximum efficiency of photosystem II (PSII) photochemistry in dark-adapted state (F_v/F_m), actual efficiency of PSII photochemistry (Φ_{PSII}) and relative water content (RWC; -2, -18 and -4%, under 94, 127 and 90 ppb, respectively). Among biochemical traits, O_3 reduced SPAD (i.e., 'leaf greenness') by 10% (under 42 ppb), whereas increased malondialdehyde (MDA; +12%, under 72 ppb), superoxide anion (O_2^-; +12%, under 200 ppb), phenols (+26%, under 49 ppb), ethylene (+130%, under 200 ppb), carbon (C; +1%, under 68 ppb) and potassium; (K; +10%, under 61 ppb). No other O_3-induced changes were observed for the remaining parameters, with total carotenoids (Car_{TOT}), superoxide dismutase (SOD), catalase (CAT), proline and jasmonic acid (JA) showing higher variability.

3.3. Differences in O_3 effects within descriptive categories

Meta-regression outcomes for descriptive categories are shown in Table 1 and Tables S3-S5 (numbers of observations and studies adopted for parameter inclusion/exclusion in the meta-regression are reported in Tables S6-S8). Net photosynthesis decreased more in deciduous than evergreen species [-27 and -14%, respectively, but with 95% confidence intervals (CIs) overlapping; Figure 4A]. This parameter, similarly to g_s, showed different O_3 effects also across native areas: P_n decreased in all species, especially in those native to Asia-Northeastern, Europe-Mediterranean and Europe-Temperate (around -35%, under 70 ppb, as average), whereas increased in those native to North America-Southwestern (+29%, under 70 ppb); g_s decreased in species native to Asia-Northeastern and Europe-Mediterranean (-46 and -13%, under 74 and 87 ppb), increased in species native to North America-Southwestern (+73%, under 70 ppb), whereas did not change in other species. Finally, different responses were observed in terms of F_v/F_m between species native to Europe-Mediterranean and to Europe-Temperate, since only the former slightly decreased due to O_3 (-1%, under 86 ppb; Figure 4B). Plant age did not affect the effects of O_3 on the few parameters analyzed after exclusion criteria were assessed [P_n, g_s and dark respiration (R_d); Table 1].

The exposure method affected the effects of O_3 on F_v/F_m and ascorbate, since F_v/F_m did not change in studies using CEC and decreased in those using OTC (-5%, under 42 ppb), and ascorbate increased in studies using CEC (+20%, under 82 ppb) and decreased in those using FACE (-13%, under 49 ppb; Figure 5A). Furthermore, root biomass decreased in investigations comparing CF *vs* E (-12%, under 47 ppb) whereas did not change in other exposure type comparisons. Leaf biomass decreased in CF *vs* E studies (-18%, under 71 ppb), whereas increased in those comparing AMB *vs* E (+41%, under 31 ppb). Conversely, g_s only decreased in AMB *vs* E comparisons (-39%, under 89 ppb). Ascorbate increased in CF *vs* E studies (+20%, under 82 ppb) and decreased in AMB *vs* E ones (-18%, under 53 ppb). Nitrogen decreased in CF *vs* E investigations (-8%, under 86 ppb), whereas did not change in AMB *vs* E ones (Figure 5B). Although between-group heterogeneity (Q_B) was significant ($P = 0.050$) for shoot/root ratio, and the mean change from control was -11% (under 60 ppb) for AOT40 >15-25 ppm h and +14% (under 45 ppb) for AOT40 >35 ppm h, 95% CIs overlapped zero, as well as each other. P_n decreased only for AOT40s of 0-5, >25-35 and >35 ppm h (-21, -41 and -22%, under 142, 47 and 69 ppb, respectively), whereas no changes were observed for other AOT40s. F_v/F_m decreased for AOT40 >15-25 ppm h and even more for AOT40 >35 ppm h (-2 and -8%, under 104 and 83 ppb, respectively), whereas no changes were observed for AOT40s lower than 15 ppm h. Ascorbate increased for AOT40 of 0-5 ppm h (+30%, under 84 ppb) and decreased for AOT40 >35 ppm h (-18%, under 53 ppb). Glutathione decreased only for AOT40 >35 ppm h (-10%, under 49 ppb), whereas did not change for AOT40 of 0-5 ppm h (Figure 5C).

Leaf biomass increased when high CO_2 was used as additional treatment (+63%, under 51 ppb), whereas did not change when O_3 was applied alone or in combination with drought. Conversely, P_n decreased when O_3 was applied alone or in combination with high CO_2 (-27 and -24%, under 84 and 98 ppb, respectively), whereas did not change when O_3 was combined with drought (Figure 6). Stomatal conductance decreased only under O_3 alone (-24%, under 80 ppb), while no changes occurred with drought or nitrogen as additional treatments. (Figure 6). Internal CO_2 concentration (C_i), hydrogen peroxide (H_2O_2) and JA increased only under O_3 alone (+7, +19 and +87%, under 75, 126 and 200 ppb, respectively), whereas did not change when drought was used as additional treatment. Intrinsic water use efficiency (WUE_{in}) did not change under O_3 alone, but decreased when O_3 was applied with drought (-41%, under 80 ppb). Although Q_B was significant ($P = 0.002$) for non-photochemical quenching (qNP), and the mean change from control was +10% (under 116 ppb) when O_3 was applied alone and -1% (under 100 ppb) when O_3 was applied with drought, 95% CIs overlapped zero, as well as each other. Pre-dawn leaf water potential (Ψ_{LPD}) increased when O_3 was applied alone (+38%, under 80 ppb), whereas decreased when O_3 was applied with drought (-10%, under 99 ppb). Ethylene emission increased under O_3 alone and even more under O_3 with drought (+251 and +54%, under 200 ppb; Figure 6).

4. Discussion

4.1. What are the overall O_3 effects on oaks?

Despite considerable variation in the responses observed among the 40 reported studies, this meta-analysis shows overwhelming evidence of the O_3 effects on 51 growth, anatomical, biomass, physiological and biochemical parameters of 14 deciduous or evergreen oak species distributed all around the Northern Hemisphere. The unexpected raise of leaf area reported among the overall effects of O_3 on oaks might suggest an O_3-induced increase in carbon allocation into shoots, particularly into leaves. However, this response was driven by the investigation of Kitao et al. (2015), where the effects of O_3 were investigated in plants grown under elevated CO_2, which is known to commonly increase leaf area (Long et al., 2004). Indeed, shoot/root ratio did not change, and an overall O_3-induced reduction in aboveground biomass by 11% was reported in the present meta-analysis. However, no other negative impacts on growth and biomass parameters were found, in contrast with other meta-analyses focused on woody species-O_3 interaction (Wittig et al., 2009; Li et al., 2017; Feng et al., 2019), suggesting a reasonable O_3 tolerance of oaks, previously reported only for some species (e.g, Alonso et al., 2014). This was further confirmed by the increased stomatal density, a morphological adjustment that have been related to O_3 tolerance, since it contributes to improve control of pollutant diffusion inside the leaves ('avoidance' strategy, *sensu* Levitt 1980), lowering O_3 loading per stomata (Paoletti and Grulke, 2005; Fusaro et al., 2016); as well as by the optimal water status (unchanged Ψ_{LPD}) preserved under stress conditions, although a slight reduction of RWC (-4%) occurred.

Although no large impacts were observed on biomass, some impairments were found at physiological level. The literature here reviewed suggested that O_3 reduced CO_2 photo-assimilation (P_n) and transpiration (E) by stomatal limitations since g_s decreased, as well as by non-stomatal factors, such as reductions in V_{cmax} and/or chlorophyll content (actually, only SPAD index decreased, differently to Chl_{TOT}). The unchanged C_i and C_i/C_a, in concomitance with reduced g_s, likely confirmed the occurrence of non-stomatal limitations. These physiological damages have been largely reported in plants under O_3 (e.g., Guidi et al., 2016; Yang et al. 2018). Although these changes might be not dramatic for oaks health, especially considering that other gas-exchange parameters did not change (e.g. WUE_i, WUE_{in}, R_d, J_{max}), significant decreases in P_n, g_s, E and V_{cmax} indicate that rising O_3 is negatively affecting both C sequestration and water vapour transfer to the atmosphere, a phenomenon that might have a significant impact at ecological level (Wittig et al., 2007).

Photosynthetic impairments were confirmed by chlorophyll fluorescence parameters. A meta-analysis of the effects of O_3 on fluorescence parameters in woody species was provided only in Chinese species by Li et al. (2017) and in poplars by Feng et al. (2019). Results of the present meta-analysis indicated that the leaf photosynthetic light reactions capacities were significantly decreased, as the chlorophyll fluorescence parameter measured under steady light conditions (i.e.,

Φ_{PSII}, -18%) was more sensitive to O_3 than the one collected in the dark-adapted state (i.e., F_v/F_m, -2%), according to Li et al. (2017). However, both qP and qNP were not affected by O_3, confirming the tolerance of oaks to the pollutant. Changes in fluorescence under steady-state illumination may reflect down-regulation process for adjusting the production of reducing power and chemical energy to a lower demand by the Calvin-Benson cycle (related with a lower Rubisco carboxylation activity). These physiological responses to elevated O_3 commonly result in less available C, but a slight increase (1%) of this element in leaves was here reported, as well as in K. Conversely, the levels of N, as well as of P, did not change, in accordance with the meta-analysis by Li et al. (2017), but not with the one by Wittig et al. (2009), where an O_3-induced increase of N was reported.

Although (partial) stomatal closure is the first response adopted by leaves in the presence of O_3 to limit its uptake, plants have also developed enzymatic and non-enzymatic antioxidant systems to cope with the oxidative pressure due to the extensive amount of reactive oxygen species (ROS) generated by the rapid degradation of O_3 reaching the apoplast (Gill and Tuteja, 2010; Döring et al. 2020). A significant increase in the lipid peroxidation marker MDA and in $^{\bullet}O_2^-$ suggested that lipid molecules were degraded by an O_3-induced overproduction of ROS, although H_2O_2 levels did not change (Pistelli et al., 2019; Podda et al., 2019). However, the enzymatic (i.e., SOD and CAT, that showed a large variability) and most of the non-enzymatic (e.g., Car_{TOT}, ascorbate, glutathione and proline) antioxidants were not affected by O_3. Only phenols increased, suggesting a key role of these compounds in oak defense to O_3. Phenols are well suited to constitute a pivotal antioxidant system with a central role in plant defense against severe constraints by avoiding the generation of ROS and by quenching them once they are formed (Gill and Tuteja, 2010). However, flavonoids, known to be secondary metabolites with high ROS scavenging ability (Agati et al., 2012), did not change.

Differently to chronic O_3 exposure, which most of the results reported above are referred to, the acute O_3 exposure (i.e., high concentrations for a very short time, such as a few hours) has been shown to result in the activation of programmed cell death (PCD) responses regulated by a cross-talk among ROS, several hormones (e.g., ABA, ethylene) and other signaling molecules (e.g., SA, JA and proline; Pellegrini et al., 2016). A meta-analytical review of the effects of acute O_3 in woody species was provided here for the first time. Overall, only ethylene resulted increased by acute O_3 (i.e., 200 ppb for 5 h), a response severely triggered (+130%) likely for signaling propagation needed in PCD (Tamaoki et al., 2003). However, these responses are highly variable within the first hours of exposure, and further studies would be needed on this interesting O_3-tree interaction(s) (and not only in oak species).

Taken together, these results pioneering demonstrate the overall responses of oaks to O_3 concentrations projected for the coming decades in some polluted areas of the Northern Hemisphere (IPCC, 2014; Verstraeten, 2015), considering that the mean O_3 concentration, averaged among the investigated parameters, resulted of around 90 ppb, ranging from 12 to 200 ppb, with exposures extending from few hours to around two years.

4.2. Do the O_3 effects differ for plant type, species native area, plant age, exposure comparison type, exposure method, O_3 severity and presence of additional treatments?

Photosynthesis is the major plant process that is severely affected by air pollutants, primarily through stomatal and mesophyll limitations (Ainsworth, 2017), and for this reason leaf CO_2 photo-assimilation is commonly used as a marker of O_3 sensitivity in plants (Wittig et al., 2007). Although only P_n showed a significant difference between plant types, this parameter suggested a higher O_3 tolerance of evergreen than deciduous species. This is consistent with the previous meta-analysis focused on Chinese woody species (Li et al., 2017), as well as with previous studies comparing Mediterranean evergreen and deciduous shrub species (Calatayud et al., 2010), and also other studies comparing different deciduous species among them: plants with smaller, thicker and more coriaceous leaves, cope better with O_3 (Calatayud et al., 2007; Bussotti, 2008). Furthermore, photosynthesis responses allowed identifying a higher O_3 sensitivity of oaks native to Asia and

Europe than those native to North America. Actually, oaks native to North America-Southwestern reported higher P_n values under increased O_3, compared with controls, and these P_n trends were positively related with those of g_s. However, this unexpected response was driven by studies focused on the responses of plants grown under O_3 to abrupt changes in environmental conditions (Grulke et al., 2007, Paoletti and Grulke, 2010). Higher g_s and P_n were interpreted as a reduced stomatal closure responsiveness (i.e., sluggishness) caused by O_3, twinned with an effective detoxification mechanism able to temporarily maintain an optimal photosynthesis level, but reported as a cause of increased O_3 sensitivity since it implies higher O_3 uptake before reaching equilibrium (Paoletti and Grulke, 2010). Furthermore, F_v/F_m, a parameter since long considered as a measurement of plant health status (Björkman and Demming, 1987), seemed to suggest a slight higher O_3 sensitivity of species native to Europe-Mediterranean (decreased by 2%) than those from Europe-Temperate areas (no change), but this difference was likely due to the higher variability of F_v/F_m reported for species native to Europe-Temperate (for which the mean decrease was higher than in Mediterranean). Plants growing in the Mediterranean area are indeed considered more tolerant to O_3 stress since are adapted to different oxidative stress factors (e.g., high temperature, strong sun-light, drought) by convergent responses (Bussotti, 2008; Calatayud et al., 2010).

Because of the difficulty of studying large and mature trees, young trees and often seedlings and saplings have formed the large part of most studies on O_3 impacts (Wittig et al., 2007). Older trees were largely under-represented in the dataset of the present study so preventing a comprehensive analysis of plant age effects. However, no significant differences in O_3 effects were here reported between young and adult trees in terms of P_n, g_s and R_d. We also found few significant differences among exposure methods, as previously reported in other meta-analyses (Wittig et al., 2007; Feng et al., 2008). However, F_v/F_m changes suggested a more detrimental O_3 effects in studies using OTC than those carried out with CEC; and an ability to activate ascorbate, which is considered the primary barrier against ROS generated from O_3 (Foyer and Noctor, 2011), was observed only in

CEC studies, whereas this antioxidant system resulted reduced in FACE ones. These responses might be caused by a lower O_3 uptake in CEC conditions since lower stomatal conductance is commonly reported under lower effective light occurring in CEC (Oksanen et al., 2005), as well as by the absence of other environmental constraints that instead might exist in less controlled conditions. Due to the limited data available, it was not possible to properly assess the differences between the effects of current (ambient air) and future (more elevated) O_3 concentrations. An effective evaluation was possible only for root biomass and g_s: root biomass suggested a more detrimental effect of future O_3 scenarios, whereas no differences among exposures were reported for g_s. However, the decreased leaf biomass and N, and the increased ascorbate reported for CF *vs* E comparison, together with the opposite trends observed when comparing AMB *vs* E, suggested that O_3 is impacting oaks already at current concentrations, but exacerbated effects will be likely caused by concentrations projected for the future, in accordance with previous meta-analysis in both tree (Wittig et al., 2007, 2009; Feng et al. 2019) and crop species (Feng et al., 2009). Differences among O_3 effects due to exposure concentrations have also been shown by the comparative analysis among AOT 40 classes (AOT40 is an O_3 exposure index widely applied for risk assessment (Lefohn et al., 2018), taking into account both the concentration of O_3 and the exposure time to this pollutant). Higher AOT40s (i.e., >25-35 and >35 ppm h) caused more detrimental effects than lower ones in terms of shoot/root ratio, P_n, F_v/F_m, ascorbate (it resulted activated by lower AOT40 of 0-5 ppm h) and glutathione. Such high AOT40 values >25 ppm h are common in Asia (especially in China; Li et al., 2017) and Southern and Central Europe (EEA, 2019). Actually, a P_n reduction was observed also for lower AOT40 class of 0-5 ppm h, likely caused by acute O_3 exposure (142 ppb for few hours), suggesting that also the effects of O_3 peaks commonly occurring across the Northern Hemisphere (IPCC, 2014) should be accurately considered.

Elucidations of the interaction of O_3 with other changing climatic variables such as drought, elevated CO_2 concentrations, temperature, salinity and low N availability are essential to develop more accurate projections of the impacts of O_3 pollution on forest trees (Wittig et al., 2007). Most of the reviewed studies focused on the interaction between O_3 and drought, whereas it was not possible to properly assess the O_3-salinity interaction due to the limited data available. The parameters showing significant differences between O_3 effects on plants under optimal water conditions or water stress (i.e., P_n, g_s, C_i, Ψ_{LPD}, H_2O_2, ethylene and JA) suggested that the constraints induced by water limitations were not further exacerbated by chronic or acute O_3, since negative effects were observed when O_3 was applied alone, and not in plants already water stressed. Only WUE_{in} was reduced by O_3 in droughted plants, conversely to those well-watered, evidencing how the gas pollutant could negatively affect this key physiological regulation adopted by plants to cope with water limitation (Yi et al., 2018). Finally, an O_3-induced increase of leaf biomass was observed in plants exposed to elevated CO_2, in contrast to those exposed only to O_3; no differences were observed in O_3 effects on photosynthesis in relation to the CO_2 levels; and g_s reduction observed in plants exposed to O_3 alone was not reported in individuals previously treated by N addition. Nevertheless, interactions between O_3 and other environmental constraints should be more deeply investigated, especially considering that some important climate change stressors such as elevated temperature have not been taken into account so far, and the interaction of the pollutant with biotic stressors has been understudied as well (Paoletti et al., 2007).

5. Conclusions

The present study provided a quantitative understanding of how O_3 pollution impair oaks. Although no large impacts were observed on biomass, suggesting an O_3 tolerance by oaks, some impairments were found at physiological level that might negatively affect both C sequestration and water vapour transfer to the atmosphere. This outcome suggested the need to fully incorporate this phenomenon into future projections of how atmospheric change and forest biomes will interact in effecting future climatic change. Among the antioxidants put in field by oaks to cope with the oxidative pressure induced by O_3, phenols seemed to have a key role. Ozone effects were more severe in deciduous species, as well as in oaks native from Asia and Europe. Experiments performed in less controlled environment showed more O_3 deleterious effects, especially when higher AOT40 levels were taken into account, but also acute O_3 exposures showed negatively impact oaks. Most of the reviewed studies including additional treatments to O_3 exposure focused on the interaction between O_3 and drought, but the negative effects induced by water deprivation on oaks seemed not exacerbated by the exposure to the pollutant. However, more combined experiments on the impact of O_3 and co-occurring stressors on woody species are necessary, since the present knowledge is insufficient. Another major issue emerged by the present study is the lack of experiments on adult trees. As previously reported (Cailleret et al., 2017), to better understand O_3 impacts at each ecosystem level and feedbacks across levels, and to reinforce the strength of O_3 impact predictions, O_3 controlled experiments on young individuals should be combined with long-term experiments on mature trees grown in open-air conditions.

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Conflict of interest

The author declares no conflict of interest.

Data availability statement

Reviewed literature is listed in Appendix A. Database of the ozone effects on oaks, details of meta-analysis and meta-regression, and numbers of observations and studies within levels of descriptive categories are included in the Supporting Information online.

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Appendix A. Articles used in the present meta-analysis of ozone impacts on oaks.

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Table 1. Between-group heterogeneity (Q_B), degrees of freedom (*df*) and *P*-value (*P*) for ozone (O_3) effect size on growth, anatomical, biomass, physiological and biochemical parameters of oaks, across descriptive categories [plant type (evergreen and deciduous), native area of species (Asia-Northeastern, Europe-Mediterranean, Europe-Temperate, North America-Central Eastern and North America-Southwestern), plant age (young and adult), exposure method (Closed Exposure Chamber, Solardome; Open Top Chamber, Branch Chamber, and Free Air Controlled Exposure, FACE), exposure type comparison (filtered air *vs* elevated O_3, filtered air *vs* ambient air, and ambient air *vs* elevated O_3), AOT40 over the entire experiment (five classes were determined: 0–5, >5–15, >15–25, >25–35, >35 ppm h), and additional treatments (no additional treatments, drought, high CO_2, nitrogen and salinity)]. Significant *P*-values are shown in bold. Data include comparisons between plants exposed to charcoal-filtered air (control) and to ambient air or increased O_3 (elevated), as well as between plants exposed to ambient air (control) and to increased O_3 (elevated). N.A.: not available (i.e. not included in the meta-regression after exclusion criteria were assessed). See Figure 3 caption for abbreviations of parameters.

Parameter	Plant type			Native area			Plant age			Exposure method			Exposure type comparison			AOT 40 class			Additional treatment		
	Q_B	*df*	*P*	Q_B	*df*	*P*	Q_B	*df*	*P*	Q_B	*df*	*P*	Q_B	*df*	*P*	Q_B	*df*	*P*	Q_B	*df*	*P*
Height		N.A.			N.A.			N.A.			N.A.			N.A.		2.00	1	0.157		N.A.	
Leaf area		N.A.			N.A.			N.A.			N.A.			N.A.		0.02	1	0.886	3.52	1	0.061
LMA		N.A.			N.A.			N.A.			N.A.			N.A.		0.51	1	0.476		N.A.	
Leaf thickness		N.A.			N.A.			N.A.			N.A.			N.A.			N.A.		0.46	1	0.117
Root biomass	0.12	1	0.727	1.00	1	0.317		N.A.			N.A.		11.30	2	**0.003**	0.57	2	0.752	0.69	1	0.406
Stem biomass	1.64	1	0.200	0.05	1	0.825		N.A.			N.A.		0.27	1	0.607	0.01	1	0.929		N.A.	
Leaf biomass	0.01	1	0.911	0.11	2	0.948		N.A.		0.00	1	0.998	9.93	1	**0.002**		N.A.		7.25	2	**0.027**
Total biomass	0.00	1	0.987	0.76	2	0.685		N.A.		2.03	1	0.154	1.74	1	0.187	0.69	3	0.875	7.28	2	0.263
Shoot/root	3.69	1	0.055		N.A.			N.A.			N.A.			N.A.		3.85	1	**0.050**	0.25	1	0.619
P_n	4.43	1	**0.035**	14.70	4	**0.005**	0.12	1	0.733	1.34	2	0.511	3.74	1	0.053	15.00	4	**0.005**	11.80	2	**0.003**
g_s	0.09	1	0.765	11.30	4	**0.023**	0.21	1	0.649	1.46	3	0.691	7.09	2	**0.029**	8.49	4	0.075	21.70	2	**0.000**
C_i	0.01	1	0.928	3.23	1	0.073		N.A.		2.87	1	0.090	0.16	1	0.689	3.80	2	0.149	5.59	1	**0.018**
WUE_{in}	0.01	1	0.932		N.A.			N.A.			N.A.			N.A.			N.A.		6.30	1	**0.012**
R_d	0.02	1	0.875	2.21	1	0.137	0.72	1	0.397	0.91	1	0.340	0.91	1	0.340		N.A.			N.A.	
V_{cmax}	0.01	1	0.910	0.19	1	0.664		N.A.		0.02	1	0.884		N.A.			N.A.		0.33	1	0.569
F_v/F_m	0.89	1	0.346	4.24	1	**0.040**		N.A.		4.07	1	**0.044**	1.61	1	0.204	12.90	3	**0.005**	1.97	1	0.160
PSII	0.60	1	0.438		N.A.			N.A.			N.A.			N.A.		0.00	1	0.963	0.87	1	0.352
qNP	0.52	1	0.470		N.A.			N.A.			N.A.			N.A.		1.06	1	0.304	9.81	1	**0.002**

Ψ_{LPD}	0.03	1	0.852		N.A.			N.A.			N.A.			N.A.			N.A.		34.40	1	**<0.001**
Chl_{TOT}	0.27	1	0.605		N.A.			N.A.			N.A.			N.A.			N.A.		0.18	1	0.672
MDA	3.50	1	0.061		N.A.			N.A.		0.19	1	0.664	0.71	1	0.401	1.11	1	0.291	0.02	1	0.894
H_2O_2	1.03	1	0.311		N.A.			N.A.		0.01	1	0.929	0.01	1	0.929	0.38	1	0.536	8.03	1	**0.005**
$^{\bullet}O_2^-$		N.A.			N.A.			N.A.			N.A.			N.A.			N.A.		0.99	1	0.320
Ascorbate	3.12	1	0.077	0.29	1	0.587		N.A.		9.42	1	**0.002**	11.20	1	**0.001**	12.40	1	**<0.001**		N.A.	
Glutathione	2.51	1	0.113	2.69	1	0.101		N.A.		2.78	1	0.095	2.78	1	0.095	5.82	1	**0.016**		N.A.	
Proline	3.78	1	0.052		N.A.			N.A.			N.A.			N.A.			N.A.		0.54	1	0.463
ABA	2.28	1	0.131		N.A.			N.A.			N.A.			N.A.			N.A.		0.01	1	0.907
Ethylene		N.A.			N.A.			N.A.			N.A.			N.A.			N.A.		4.20	1	**0.040**
SA		N.A.			N.A.			N.A.			N.A.			N.A.			N.A.		1.46	1	0.227
JA		N.A.			N.A.			N.A.			N.A.			N.A.			N.A.		6.76	1	**0.009**
N	0.25	1	0.621	2.44	2	0.295		N.A.		1.32	1	0.250	4.02	1	**0.045**		N.A.		0.29	1	0.587

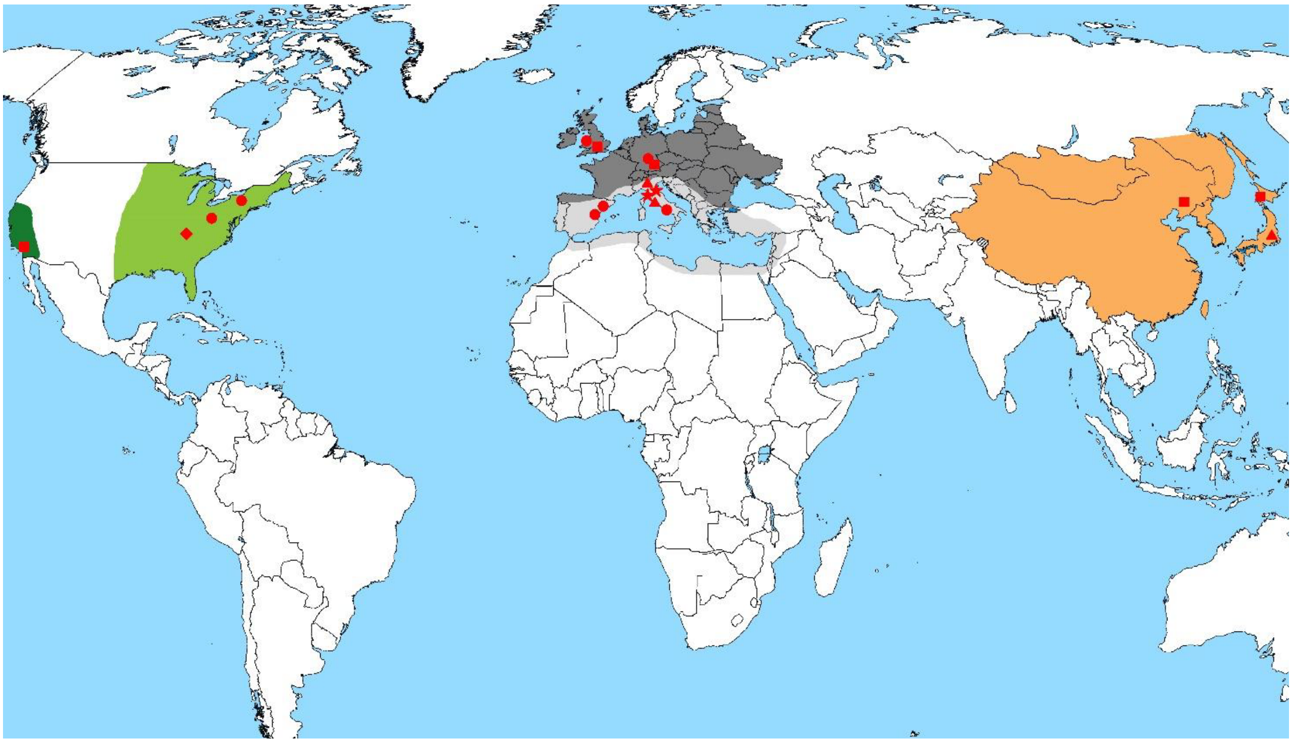

Figure 1. Global distribution of experimental sites of the studies included in the present meta-analysis. Symbols represent the number of studies performed in each experimental site: one (circle), two (square), three (triangle), four (diamond), five (star). Native areas of oak species included in the present meta-analysis (see Figure 2) are also reported with different colors: Asia-Northeastern (orange), Europe-Mediterranean (light gray), Europe-Temperate (dark gray), North America-Central Eastern (light green) and North America-Southwestern (dark green).

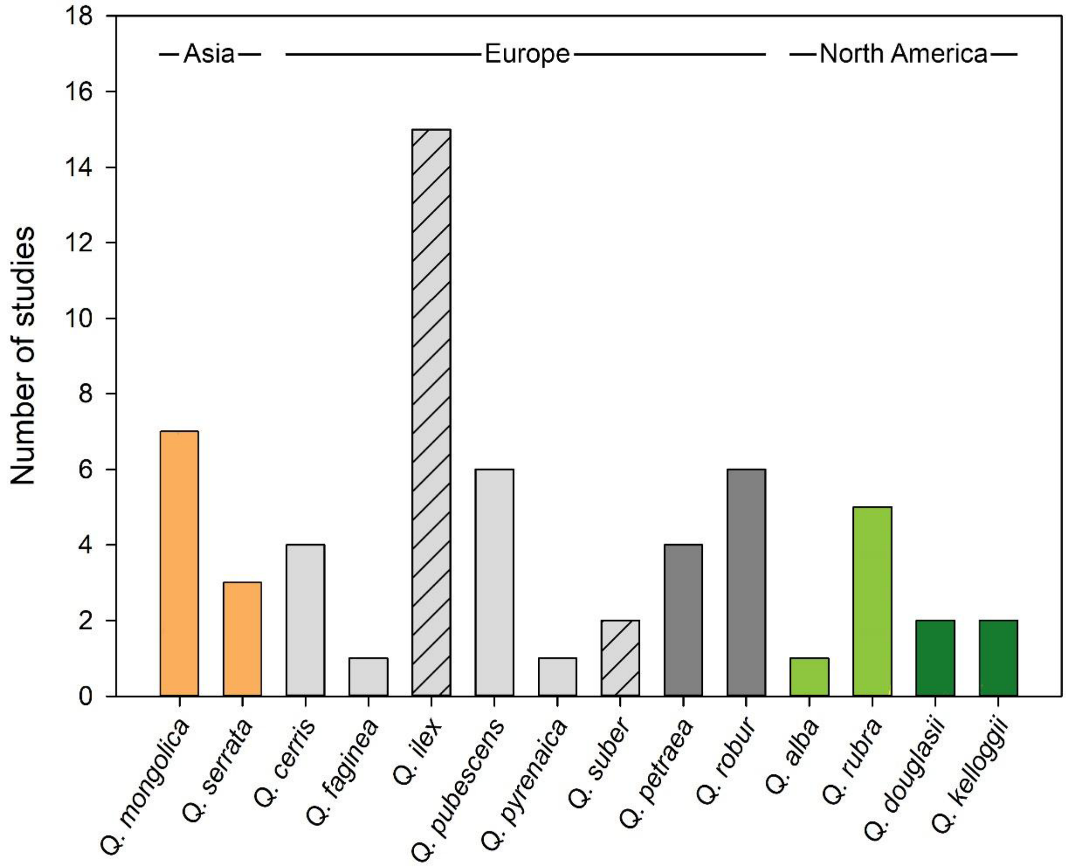

Figure 2. Number of studies used in the present meta-analysis including oak species native to different global areas: Asia-Northeastern (orange), Europe-Mediterranean (light gray), Europe-Temperate (dark gray), North America-Central Eastern (light green) and North America-Southwestern (dark green). Evergreen species are patterned, unlike deciduous.

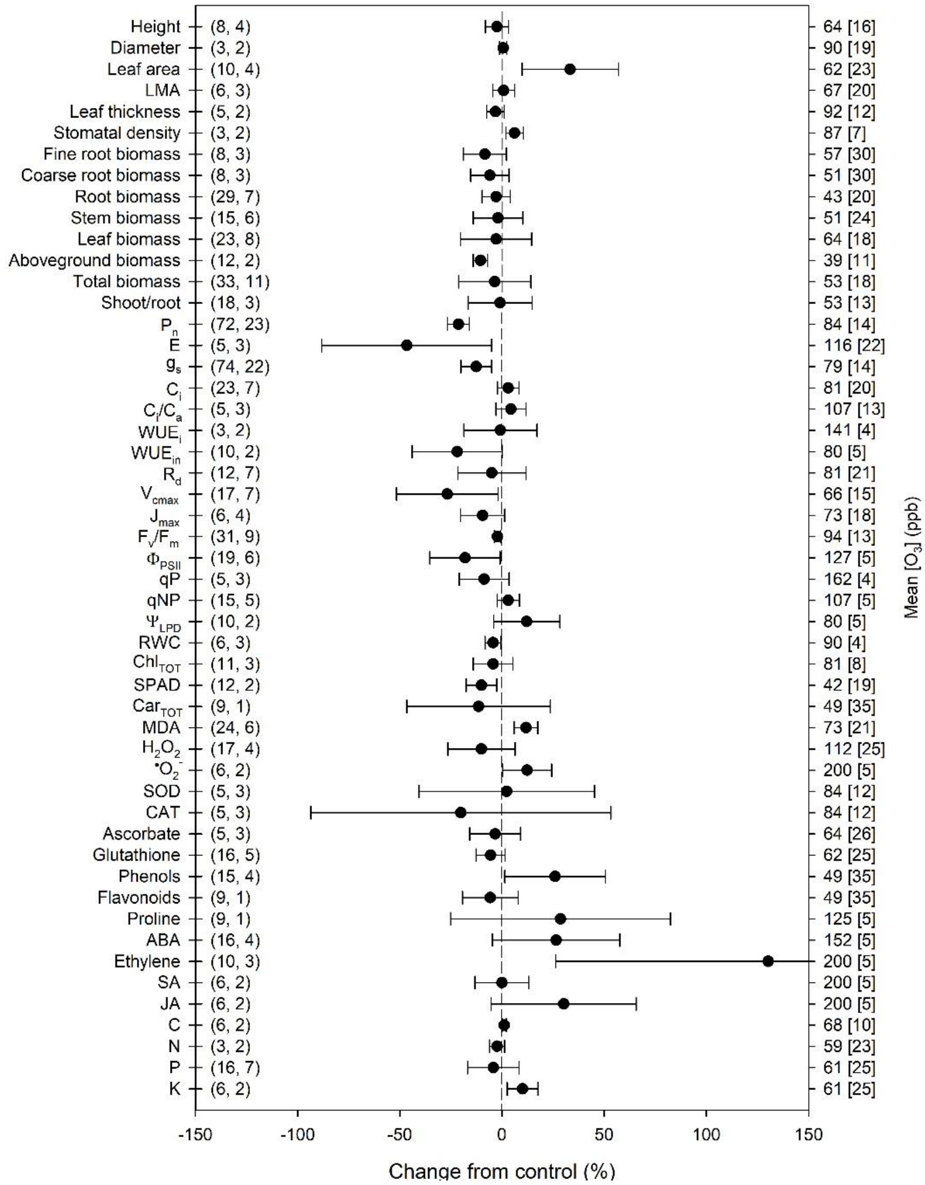

Figure 3. Effects of ozone (O_3) on growth, anatomical, biomass, physiological and biochemical parameters of oaks. Symbols represent the mean percent change due to elevated O_3 relative to control, and the bars show the 95% confidence interval. Data include comparisons between plants exposed to charcoal-filtered air (control) and to ambient air or increased O_3 (elevated), as well as between plants exposed to ambient air (control) and to increased O_3 (elevated). Number of measurements and papers are shown in parentheses; whereas mean elevated O_3 and control O_3 (in brackets) concentrations are given along the right *y* axis. Abbreviations: ABA, abscisic acid; C, carbon; Car_{TOT}, total carotenoid; CAT, catalase; Chl_{TOT}, total chlorophyll; C_i, intercellular carbon dioxide (CO_2) concentration; C_i/C_a, internal and ambient CO_2 concentration ratio; E, transpiration; F_v/F_m, maximum efficiency of PSII photochemistry in dark-adapted state; g_s, stomatal conductance; H_2O_2, hydrogen peroxide; JA, jasmonic acid; J_{max}, maximum rate of electron transport; K, potassium; LMA, leaf mass per area; MDA, malondialdehyde; N, nitrogen; $^{\bullet}O_2^-$, superoxide anion; P, phosphorus; P_n, net photosynthesis; qNP, non-photochemical quenching; qP, photochemical quenching; R_d, dark respiration; RWC, relative water content; SA, salicylic acid; SOD, superoxide dismutase; V_{cmax}, maximum rate of Rubisco carboxylation; WUE_i, instantaneous water use efficiency; WUE_{in}, intrinsic water use efficiency; Φ_{PSII}, actual efficiency of PSII photochemistry; Ψ_{LPD}, pre-dawn leaf water potential.

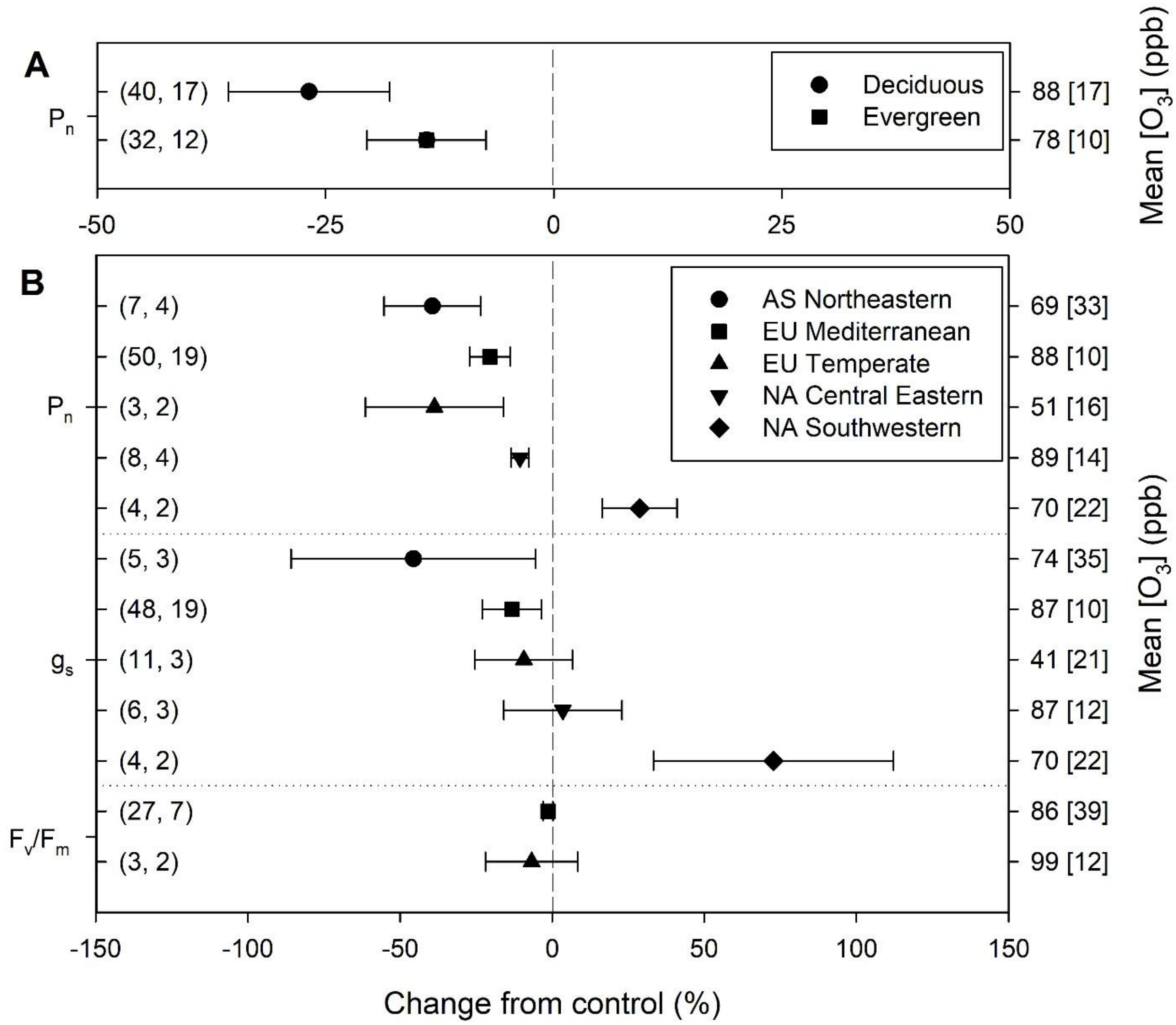

Figure 4. Differential effects of ozone (O_3) on net photosynthesis (P_n) of oaks on the basis of the plant type (deciduous and evergreen; **A**); and on P_n, stomatal conductance (g_s) and maximum efficiency of PSII photochemistry in dark-adapted state (F_v/F_m) of oaks on the basis of the native area (AS Northeastern, EU Mediterranean, EU Temperate, NA Central Eastern and NA Southwestern; **B**). Symbols represent the mean percent change due to elevated O_3 relative to control, and the bars show the 95% confidence interval. Data include comparisons between plants exposed to charcoal-filtered air (control) and to ambient air or increased O_3 (elevated), as well as between plants exposed to ambient air (control) and to increased O_3 (elevated). Number of measurements and papers are shown in parentheses; whereas mean elevated O_3 and control O_3 (in brackets) concentrations are given along the right *y* axis.

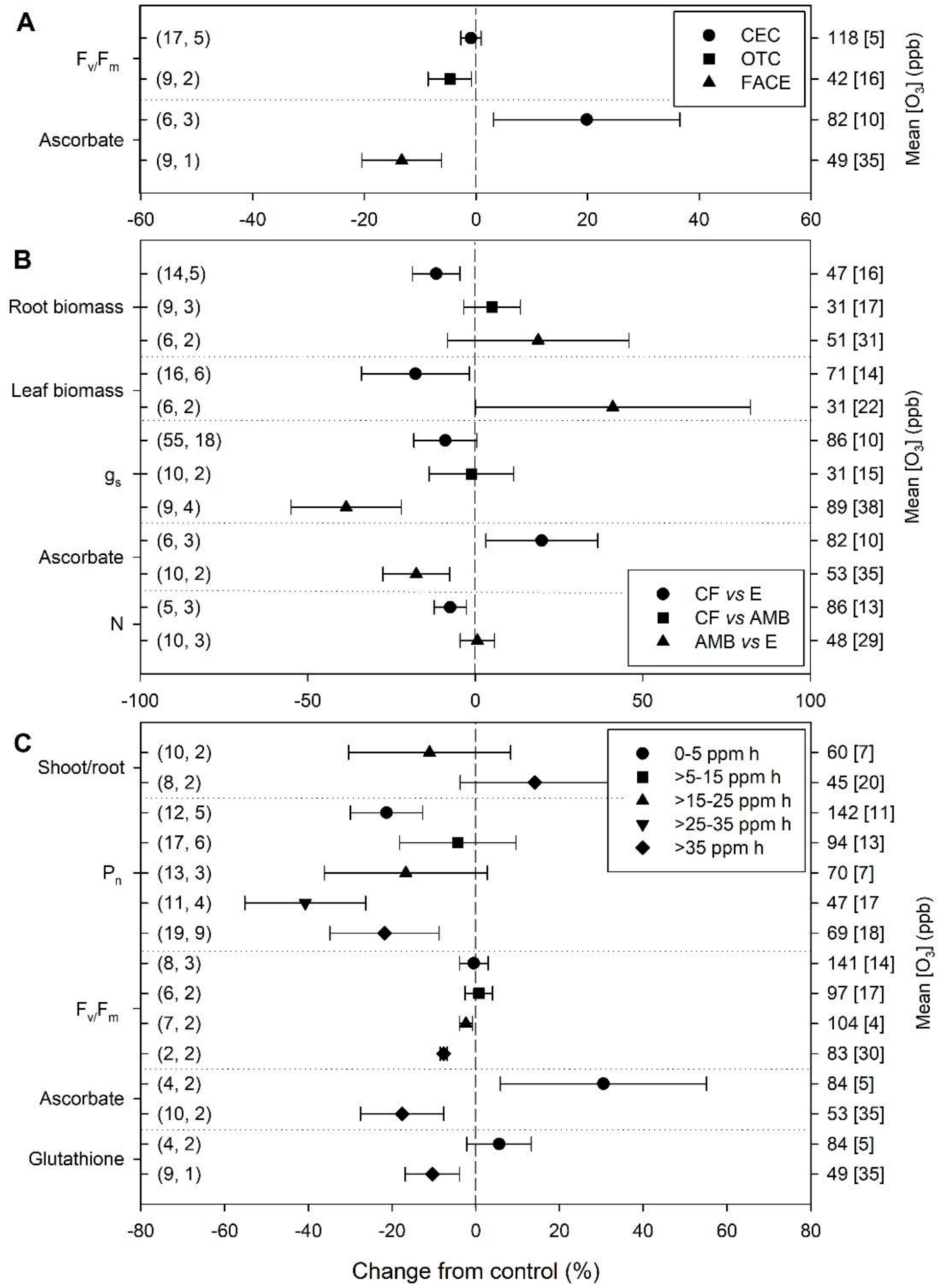

Figure 5. Differential effects of ozone (O_3) on maximum efficiency of PSII photochemistry in dark-adapted state (F_v/F_m) and ascorbate of oaks on the basis of the exposure method (Closed Exposure Chamber, CEC; Open Top Chamber, OTC; Free Air Controlled Exposure; **A**); on root and leaf biomass, stomatal conductance (g_s), ascorbate and nitrogen (N) on the basis of the exposure type comparison [charcoal-filtered air (CF) *vs* elevated O_3 (E), CF *vs* ambient air (AMB), and AMB *vs* E; **B**]; and on shoot/root ratio, net photosynthesis (P_n), F_v/F_m, ascorbate and glutathione of oaks in the basis of the AOT40 class (0–5, >5–15, >15–25, >25–35, and >35 ppm h; **C**). Symbols represent the mean percent change due to elevated O_3 relative to control, and the bars show the 95% confidence interval. Data include comparisons between plants exposed to charcoal-filtered air (control) and to ambient air or increased O_3 (elevated), as well as between plants exposed to ambient air (control) and to increased O_3 (elevated). Number of measurements and papers are shown in parentheses; whereas mean elevated O_3 and control O_3 (in brackets) concentrations are given along the right *y* axis.

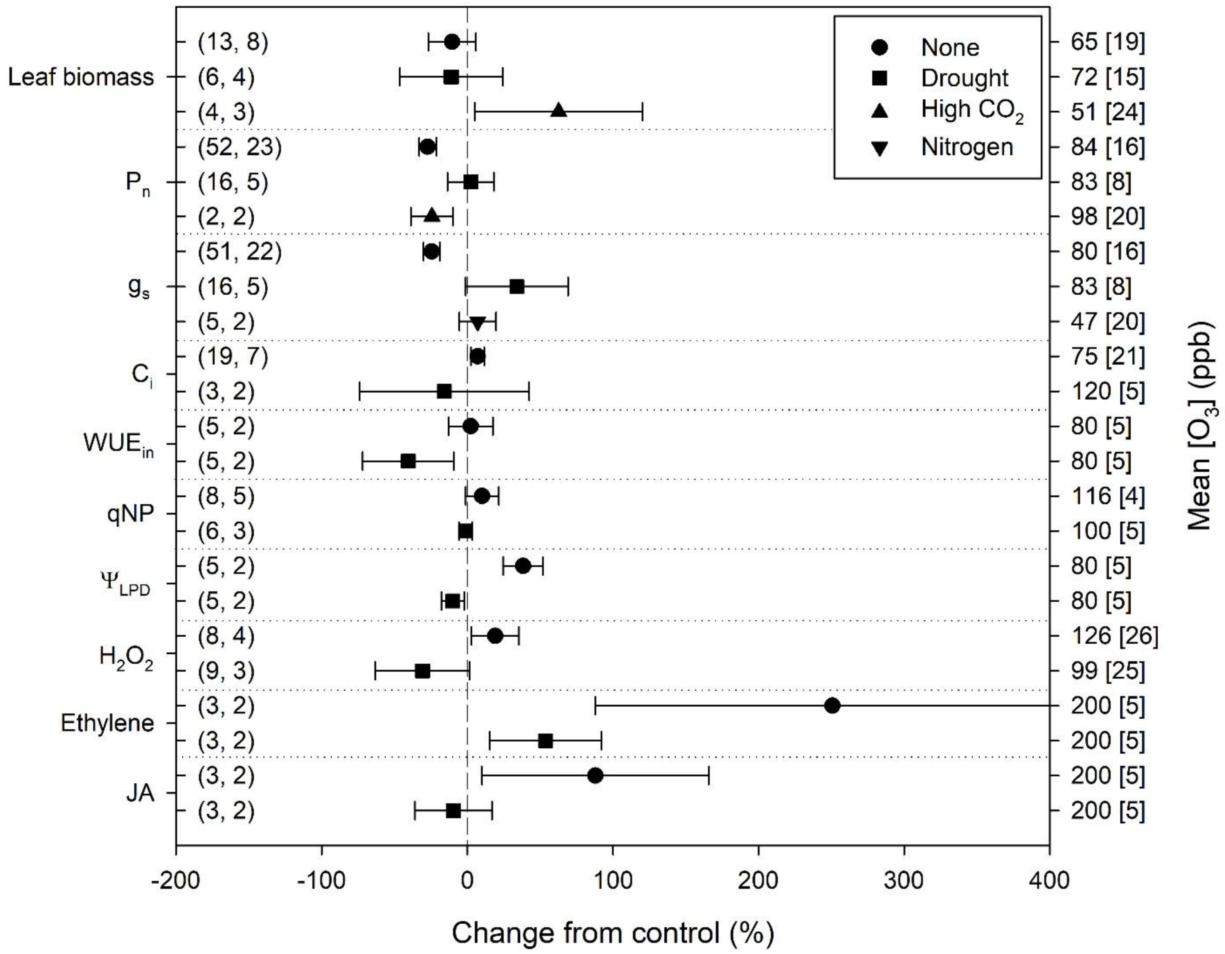

Figure 6. Differential effects of ozone (O_3) on leaf biomass, net photosynthesis (P_n), stomatal conductance (g_s), internal carbon dioxide (CO_2) concentration (C_i), intrinsic water use efficiency (WUE_{in}), non-photochemical quenching (qNP), pre-dawn leaf water potential (Ψ_{LPD}), hydrogen peroxide (H_2O_2), ethylene and jasmonic acid (JA) of oaks on the basis of the additional treatment (none, drought, high CO_2 and nitrogen). Symbols represent the mean percent change due to elevated O_3 relative to control, and the bars show the 95% confidence interval. Data include comparisons between plants exposed to charcoal-filtered air (control) and to ambient air or increased O_3 (elevated), as well as between plants exposed to ambient air (control) and to increased O_3 (elevated). Number of measurements and papers are shown in parentheses; whereas mean elevated O_3 and control O_3 (in brackets) concentrations are given along the right *y* axis.

*Declaration of Interest Statement

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☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: